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Examination of egg white proteins and effects of high pressure on select physical and functional properties

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Examination of egg white proteins and effects of high pressure on select physical and functional properties

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Examination of egg white proteins and effects of high pressure on select physical and functional properties

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Egg white proteins have become an important and desirable ingredient to the food industry due to their functional properties which include gelling, foaming, and emulsification. Egg white is also well recognized as an excellent source of nutrition. The goal of this work was to determine the effects of high pressure (HP) treatment on egg white proteins. Specifically, experiments were conducted using Raman spectroscopy and pepsin digestibility to investigate structural changes. Pressure treatment at 400 to 800 MPa (5 minutes at 4°C) resulted in increased pepsin digestibility of egg white proteins ovalbumin, ovotransferrin, and lysozyme compared to heat-treated (85 to 95°C) and untreated controls. Increased digestibility was also evident at pressures that did not result in gelation. Raman spectroscopy analysis of protein secondary structural changes resulting from HP-treatment showed an increase in β-sheet/α-helix ratio at these pressure ranges. ACE inhibitor peptide YAEERYPIL (origin ovalbumin) was identified from 800 MPa pepsin digestion sample via Liquid Chromatography/mass spectrometry/mass spectrometry (LC/MS/MS). HP-induced changes in egg white functionality were evaluated by determining foaming and gelation properties with and without prior pressure treatment. Gels were formed at pressures of 600 to 800 MPa and with heat treatment of 85°C to 95°C. HP gels were softer and more elastic than heat treated gels. Lowering the pH to 6 with tartaric acid improved overall gel appearance. With respect to foaming

properties, HP increased foam capacity while decreasing stability. Overall, HPP improved egg white functional properties and has the potential to improve egg white nutritional value through increased digestibility.

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Literature Review

Introduction

Egg white is a well recognized functional and nutritional food ingredient. The recent development of non-thermal technology employing high hydrostatic pressure processing (HPP) has shown promise to further enhance those properties. It has also has shown promise in reduction of microorganisms and increasing product shelf life without the use of preservatives. This is in line with consumer demands for safe and preservative free or natural, minimally processed foods (Rastogi, N.K et al 2007). Thus, it is essential to study the effects of HPP on egg white protein and evaluate the impact it has on egg white as a functional food ingredient.

It is also important to determine the effects of HPP on whole egg white digestibility. Egg white is well recognized as an excellent nutrition source and it is likely that pressure induced denaturation will increase digestibility without the side effects of thermal processing. This study was focused on expanding the understanding of the effect of HPP on egg white proteins. The following literature review specifically discusses the composition of egg white, HPP technology, protein structure and digestibility, and potential nutritional effects with respect to allergenicity and bioactive peptides. The factors affecting egg white functional properties of foaming and gelation are also discussed.

Objectives

The following outlined objectives were used to test the hypothesis that high pressure (HP) treatment will result in protein denaturation and increased pepsin digestibility. Secondly, HP treatment should result in the formation of gels without heat

and a shift in protein secondary structure from α-helix to β-sheet. Thirdly, HP treatment should increase the foaming ability of egg white. Finally, the nutritional value of egg white may be increased due to protein denaturation and release of potentially bioactive peptides. The goal of this work was to examine the effects of HPP on whole egg white protein functionality and digestibility, in greater detail. Specifically, the objectives of this work were to investigate:

1. HP-induced changes in egg white protein secondary structure using Raman spectroscopy
2. The effects of HP treatment on egg white protein pepsin digestibility
3. The peptide products of pepsin digested HP-treated egg white and identify bioactive peptides based on sequence identity
4. Effects of HP treatment on gelation, texture, foaming, and color of egg white

Egg Composition

The egg has long been known for its exceptional nutritional value. It consists of a porous carbonate shell, yolk, and albumen commonly known as egg white. The yolk makes up 1/3 of the egg and contains most of the vitamins including A, D, E, K, and B-complex vitamins. The yolk also contains essentially all of the lipids, ¾ of the calories, and is a good source of antioxidant carotenoids. In contrast, egg white contains over half of the proteins in egg and is a source of the vitamin riboflavin (Mine, Y. et al 2006). Egg whites are low in lipids at 0.01% (Mine, Y. et al 1995), making egg white a healthy source of protein and other nutrients.

Egg white is composed of ~9.7-10.6% protein by weight. Over 24 different proteins have been identified and isolated from egg white (Mine,Y. et al 2006). Some of

the major proteins include ovalbumin (54%), ovotransferrin (12%), ovomucoid (11%), ovomucin (3.5%), and lysozyme (3.4%) (Mine,Y. et al 1995). The following table lists selected properties of the major egg white proteins (Table 1).

Table 1: Major egg white proteins and selected properties (Mine, Y. et al 1995)

Protein	**% protein in egg white**	**Molecular Weight (kDa)**	**pI**	**Denaturation Temperature (°C)**
Ovalbumin	54	44.5	4.5	84.0
Ovotransferrin	12	77.7	6.1	61.0
Ovomucoid	11	28.0	4.1	77.0
Ovomucin	3.5	$5.5\text{-}8.8\times10^{3}$	4.5-5.0	Unknown
Lysozyme	3.4	14.3	10.7	75.0

Ovalbumin

The most abundant and central protein to egg white's functional properties in foods is ovalbumin. Ovalbumin has a molecular weight of 44.5 kDa and is a monomeric phosphoglycoprotein with a known complete amino acid sequence of 385 residues (see appendix) (Doi, E. et al 1997). It is a storage protein and major source of amino acids for the developing embryo (Mine, Y. et al 2008). The N-terminus of ovalbumin is acetylated and contains four sulfhydryl groups and one disulfide bridge (Cys74-Cys121), which are inaccessible in the native state (Doi, E. et al 1997; Iametti, S. et al 1998). Although it is a secretion protein, ovalbumin is lacking an N-terminal leader sequence. Trans-membrane location is instead mediated by an internal sequence signal located within hydrophobic residues 21-47 (Huntington J. A. et al 2001; Uniprot.org, 2010). Ovalbumin secondary structure has various motifs including α-helix (41%), β-sheet (34%), β-turns (12%), and random coils (13%) (Ngarize, S. et al 2004a). The 3-D structure of ovalbumin is highly structured and has an α-helical reactive loop coming out of the main body of the protein on two peptide stocks and a main β-sheet A (**Figure 1**). The conserved reaction center is located at Ala358-Ser359 (Stein, P.E. et al 1990).

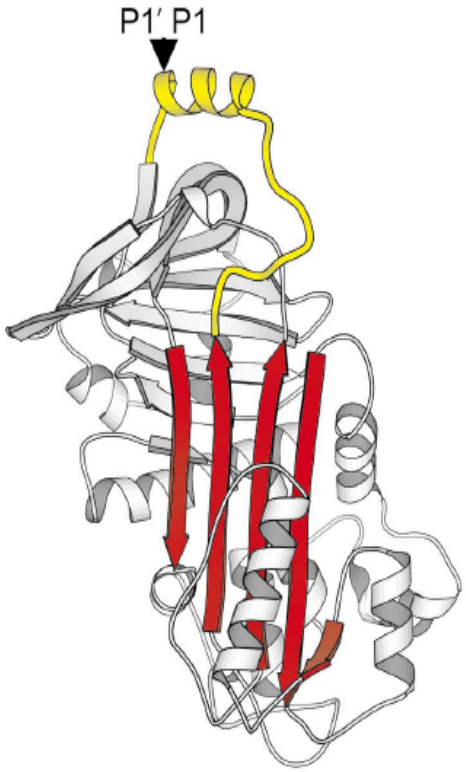

Figure 1: The 3-D crystal structure of ovalbumin with the α-helix reaction loop in yellow and main β-sheet A in red (Huntington, J. A. et al 2001).

Ovalbumin is a heterogeneous molecule with variation in its composition, which includes the degree of phosphorylation, glycosylation, and genetic variance. Two possible glycosylation sites have been identified at residues Asn 293-295 (Asn-X-Thr) and Asn 317-319 (Asn-X-Ser). The heterogeneous carbohydrate peptide chains contain a common core of mannose β (1-4) glcNAc β (1-4) glcNAc (Huntington, J. A. et al 2001). Purified ovalbumin contains three types, A_1, A_2, and A_3 in a ratio of 85:12:3. These types are differentiated by the degree of phosphorylation with two, one and zero phosphorylated sites respectively (Doi, E. et al 1997). The phosphorylation sites are located at serine residues 69 and 345. It is thought that the glutamic acids two residues C-terminal to the serine phosphorylation sites play a role in recognition for a protein kinase (Huntington, J. A. et al 2001). The degree of phosphorylation is most likely

responsible for the multiple spots observed in 2-D electrophoresis analysis of ovalbumin (Guerin-Dubiard, C. et al 2006). Genetic variance includes polymorphism substitutions at residue 290, Glu→Gln, and residue 312, Asn→Asp (Huntington, J. A. et al 2001). Ovalbumin also has X and Y genes with the Y-polymorphism occurring due to "alternative splicing processing leading to casual exon skipping events" (Guerin-Dubiard, C. et al 2006).

S-ovalbumin is found naturally in egg white and contributes to ovalbumin heterogeneity. It is an alternative form of ovalbumin with greater heat stability and is known as "stable" ovalbumin. The presence of S-ovalbumin is confirmed by the difference in denaturation temperature at 92.5°C compared to 84.5°C for ovalbumin. Other properties of S-ovalbumin such as molecular weight, sulfhydryl content, crystal formation, and electrophoretic separation are indistinguishable from ovalbumin. However, a slightly more compact structure has been observed by Raman difference spectroscopy (Doi, E. et al 1997). The more compact structure may contribute to its heat stability. S-ovalbumin has also been found to have increased surface hydrophobicity (Kilara, A. et al 1996). The mechanism for conversion of ovalbumin to S-ovalbumin has not been confirmed but may be a result of deamidation or partial reactive loop insertion (Doi, E. et al 1997; Huntington, J. A. et al 2001). S-ovalbumin content in egg white increases with age and can be as low as 5% in fresh egg to 81% after 6 months at 2°C (Kilara, A. et al 1996). The crystal structure of S-ovalbumin has been determined (Yamasaki, M. et al 2003) and shows no difference in secondary structure with ovalbumin. Some differences include a switch from the L to D isomers of Ser residues 164, 236, and 320 along with a separation in a β-strand between residues 125-128. These

differences decrease the solvent access to the protein core, contributing to increased stability.

The amino acid sequence and 3D structure of ovalbumin show similarities to a group of serine protease inhibitors known as serpins. However, ovalbumin does not have inhibitory activity (Doi, E. et al 1997). The crystal structure of ovalbumin has been used as a model for an un-cleaved reactive center of serpins (Stein, P. E. et al 1990). The serpin family consists of over 300 different proteins, with most serving a simple function such as human plasma proteins that control coagulation (Huntington, J. A. et al 2001). Serpins share a highly ordered structure and a conserved reactive center (Stein, P. E. et al 1990). Like ovalbumin, the reactive center is protruded out of the main protein body on peptide “stalks”. When a serpin comes in contact with a protease it activates by undergoing a conformational change where the reactive center loop is cleaved and inserted in β-sheet A. This conversion is thermodynamically favorable and the resulting conformation is up to twice as stable as the native form (Huntington, J. A. et al 2001). The following figure illustrates a serpin protein in native and activated conformation (**Figure 2**).

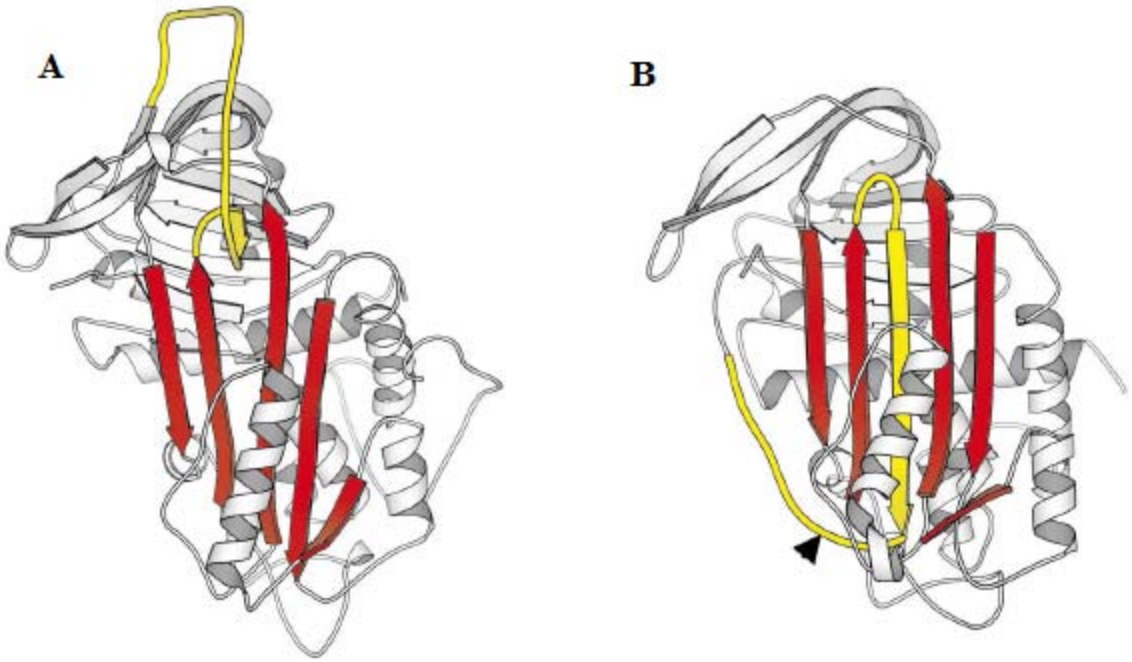

Figure 2: The crystal structures of native antithrombin (A) and activated antithrombin (B). The reactive center loop is in yellow. The reactive loop is inserted in β-sheet A shown in red Huntington, J. A. et al 2001).

Ovalbumin does not undergo this conformational change upon cleavage of its reactive loop which is the primary explanation why ovalbumin is not inhibitory. The conformational change is dependent on the successful insertion of the reactive loop hinge region, labeled P15-P8 (ovalbumin sequence 338-346). This involves alternating side chains of the hinge region being buried in the hydrophobic core of the protein. Serpins have a highly conserved hinge region, consisting of small hydrophobic and amphipathic amino acids. P14 is the first amino acid inserted followed by P12 and are conserved as threonine (80%) and alanine (98%) in inhibitory serpins, respectively. These small side chains are one driving force for the conformational change. In contrast, ovalbumin has a bulky and charged arginine at P14 and valine at P12. This is detrimental to loop insertion as it is thermodynamically unfavorable due to a loss of hinge flexibility. The cleavage of the reactive loop in ovalbumin actually results in a loss of 1-2°C in stability. However

this is only a partial reason for lack of inhibition, as switching P14 from threonine to arginine in an inhibitory serpin still results in loop insertion but with reduced inhibitory activity (Huntington, J. A. et al 2001; Stein, P. E. et al 1990).

Ovotransferrin

Ovotransferrin is the second most abundant egg white protein, accounting for 12% of protein in egg white. It has a molecular weight of 77.7 kDa with a pI of 6.1 and is a glycoprotein consisting of 686 amino acid residues (Mine, Y. et al 1995). Ovotransferrin is a member of an iron binding protein group known as transferrins. Its iron-binding activity, $K_D = 10^{-29}$M (Kilara, A. et al 1996), is thought to be responsible for the antimicrobial properties of the protein. Up to 2 Fe^{+3} and CO_3^{-2} ions can bind per molecule. The 3D structure of ovotransferrin has been determined and consists of two homologous lobes (N-lobe and C-lobe) in which each lobe has two domains. The iron-binding sites are located between these domains and include Asp63, Tyr95, Tyr188, His249 and Asp392, Tyr426, Tyr517, His585 in the N and C lobe respectively (Nakamura, R. et al 2000). The metal binding action helps to stabilize the protein raising the denaturation temperature from 61°C to around 72°C when iron is bound (Kilara, A. et al 1996). Ovotransferrin contains 15 disulfide bridges with 6 in the N-lobe and 9 in the C-lobe (Nakamura, R. et al 2000). The lone glycan chain is composed of mannose and N-acetyglucosamine and is located in the C-terminal lobe (Mine, Y. et al 1995).

Ovomucoid

Ovomucoid is a glycoprotein with a molecular weight of 28.0 kDa and pI of 4.1. About 25% of the protein is carbohydrates that are attached via Asp residues. There are 9 disulfide bridges and no free sulfhydryl groups. Ovomucoid is a well known trypsin

inhibitor (Mine, Y. et al 1995), with a 1:1 K_D of 1.5×10^{-7} M (Kilara, A. et al 1996). The 3D structure has 3 domains which are cross-linked via disulfide bonds. The domains are homologous to pancreatic secretory trypsin inhibitor. The trypsin inhibitor reactive site is located in domain 2 (Arg89-Ala90). Domain 1 and 2 each have N-terminal carbohydrate chains while domain 3 can be without a carbohydrate chain. The chains consist of pentaantennary and tetraantennary complexes with mannose, galactose, and N-acetylglucosamine. Ovomucoid's secondary structure includes 26% α-helix, 46% β-sheet, 10% β-turns, and 18% random coils (Nakamura, R. et al 2000). Ovomucoid is very stable due to its multiple disulfide bridges and is physicochemical unchanged under acidic conditions at 100°C for long periods of time (Kilara, A. et al 1996). However with extreme heat, trypsin inhibitory activity and immunoreactivity with some antibodies is lost due to the reduction and alkylation of disulfide bonds (Nakamura, R. et al 2000).

Ovomucin

Ovomucin is a viscous glycoprotein that composes 1.5-3.5% of protein in egg white. Its molecular weight ranges between $5.5\text{-}8.8 \times 10^3$ kDa and a pI of 4-5.5 (Mine, Y. et al 1995). Ovomucin is insoluble in water unless in the presence of salt or >pH 9 (Nakamura, R. et al 2000). It consists of a carbohydrate poor form, α-ovomucin, and carbohydrate rich form, β-ovomucin. The two forms complex to form an insoluble thick egg white and a combination thick and thin egg white (Mine, Y. et al 1995). Insoluble egg white or thick egg white has a ratio of 84:20 α/β forms while the soluble egg white or thin ratio is 40:3. The carbohydrate content of α-ovomucin and β-ovomucin are ~15% and ~50%, respectively. The carbohydrate chains are 15-18.6% hexose, 7-12% hexosamine, and 2.5-8% sialic acid (Nakamura, R. et al 2000). Ovomucin is an inhibitor

of virus hemagglutination and is an important determinant for egg quality (Kilara, A. et al 1996) as the thinning of egg white is thought to be caused by dissociation of α-ovomucin from insoluble ovomucin (Nakamura, R. et al 2000).

Lysozyme

Lysozyme was the first protein to be sequenced and is one of the most studied egg white proteins. It is a small protein, consisting of 129 amino acids with a molecular weight of 14.3 kDa and a pI of 10.7. Lysozyme contains 4 disulfide bridges with no free sulfhydryl groups and its 3D structure has been determined (Lesnierowski, G. et al 2007). With a similar 3D structure and 40% sequence homology to the milk protein α-lactalbumin, it is possible that lysozyme and α-lactalbumin evolved from a common protein (Nakamura, R. et al 2000). Lysozyme contains two domains connected by a long α-helix. The N-terminal domain is mostly made up of anti-parallel β-sheet with a few α-helices, while the other domain is mostly α-helical (Lesnierowski, G. et al 2007).

Lysosymes are a group of enzymes with antimicrobial function by lysis of gram negative bacteria. They are found in a wide range of organisms including bacteria, phages, vertebrates, and invertebrates. Type C is found in chicken egg white and is the most common form. Lysis of gram negative bacteria occurs with the hydrolysis of the β (1-4) linkage between acetylglucosamine and N-acetylmuramic acid in the cell wall (Nakamura, R. et al 2000). The helix-loop-helix motif located between lysozyme's two domains (Asp87-Arg114) plays an important role in this function. The reduction of more than 2 of the disulfide bonds results in a loss of bioactivity (Lesnierowski, G. et al 2007). However, reduction of disulfide linkages significantly improves functional properties including gelation and foaming (Doi, E. et al 1997). Along with its role in interaction

with other proteins during foaming and gelation, lysozyme may play a role in the thinning of egg white during storage through electrostatic interactions with ovomucin (Mine, Y. et al 1995).

High Pressure Processing

High Pressure Processing (HPP) provides an alternative, non-thermal method for food preservation. HPP was first extensively used industrially by Japan in the 1990s (Rovere, P. 2001) and is currently used for products such as sauces, jams, jellies, fruit juices, guacamole, and oysters. Advantages of HPP include the ability to process food at lower temperatures, reduce microorganisms, avoid use of chemical additives for preservation, and produce foods with new functional properties (Rastogi, N. K. et al 2007; San Martin, M. F. et al 2002). This is important to consumers as there is an increasing demand for products that are processed naturally, retain their nutritional value, and are shelf-stable and safe. Pressure is also applied uniformly throughout the food matrix, which eliminates the problem of uneven treatment that can occur with thermal methods. In contrast to HPP, thermal processing decreases the nutritional and sensory properties of food due to heat induced chemical reactions such as Maillard browning and irreversible protein denaturation (Ngarize, S. et al 2005; San Martin, M. F. et al 2002). As a result of its many applications and potential to produce novel food products, HPP has been subject of extensive research and review (Hendrickx, M. et al 2001).

There are also some challenges that are present with HPP. One challenge is heat transfer problems that may result in non-uniformity during processing. This is related to the fact that as pressure is applied or a product is compressed the temperature is increased. The temperature change is dependent on a number of factors including initial

temperature of the product, target pressure, heat transfer to surroundings, and product constituents. Water content is of particular importance as there is a 3°C temperature increase in water for every 100 MPa of pressure. This is also important for the pressure transmitting fluid which can transmit heat to the product under pressure. Another challenge is the overall lack of knowledge of HPP effects on various food systems and reproducibility of data due to incomplete records of processing conditions (i.e location of thermocouple in pressure vessel to measure temp changes) (Rastogi, N. K. et al 2007). Another challenge to consider is the economics of HPP as it is more expensive than conventional thermal processing methods. This is associated with the need to load and unload due to batch systems (time), cost of automation, and initial capital of HPP unit. Thus, HPP has become more commonly used for niche products (Van den Berg, R. W. et al 2001).

The two major HPP units for food applications are the batch system and flow through system. The flow through system is semi-continuous, requiring the product to be pumped (fruit juices) and is pressurized using a floating piston. Batch systems include three major components: the pressure vessel, surrounding yoke, and hydraulics. Pressure is applied in the vessel via a medium fluid which is pressurized with hydraulic pumps. The medium fluid usually consists of a water/soluble oil mixture (Van den Berg, R.W. et al 2001). Batch systems also require the product to be pre-packaged in a flexible pouch or tube before processing (Tewari, G. 2007). Depending on the HP unit, pressure is generated using either internal or external compression. With internal compression, the volume of the treatment vessel is reduced by the action of hydraulic pressure from a piston. The more common external compression is achieved by pumping the

pressurization medium into the chamber with high pressure pumps to reach the desired pressure (Martin San, M. F. et al 2002).

The structure of most food is undamaged during HPP due to isostatic pressing. The external pressure on the food is equal to the internal pressure when immersed in the pressure-transmitting medium so the product retains its original geometry. However, HPP has a significant effect on functionality and the rheological properties of a product. HPP effects on macromolecules such as proteins, carbohydrates, and lipids are responsible for most of these changes as molecular interactions are affected. Unlike thermal treatments, HPP does not affect covalent bonds such as cross linkages within macromolecules. The one exception is disulfide bonds in proteins. In starches, pressure generally raises the gelatinization temperature while increasing amylase digestibility. HPP ultimately destroys the granular structure of starches via hydration of the amorphous phase and distortion of the crystalline region. Similar to proteins, some carbohydrates form gels with HP treatment (Heremans, K. 2001). HP treatment tends to increase peroxide values of lipids resulting from oxidation. Para-anisidine values are also increased, resulting in more secondary oxidation products (Ludikhuyze, L. et al 2001).

HPP significantly affects secondary, tertiary, and quaternary structure of proteins. Changes in tertiary structure are particularly important to protein functionality (Tewari, G. 2007). The structural changes are due to the breakage of non-covalent interactions such as hydrogen bonds, hydrophobic interactions, and ion-pair bonds. Reformation of intra and inter molecular bonds results in changes in protein structure. HPP has also been shown to decrease protein surface hydrophobicity while increasing solubility of casein proteins (Rastogi, N. K. et al 2007). The level of denaturation is dependent on a number

of factors including type of protein, concentration, pH, and ionic strength. The kinetic relationship of protein denaturation as a function of temperature and pressure has been studied (Suzuki, K. 1960). This relationship is given in the figure below (**Figure 3**). The elliptical nature of the phase diagram indicates that at lower pressures the denaturation temperature of proteins increases while at higher pressures the denaturation temperature decreases. Pressure denaturation is also slower at low temperatures.

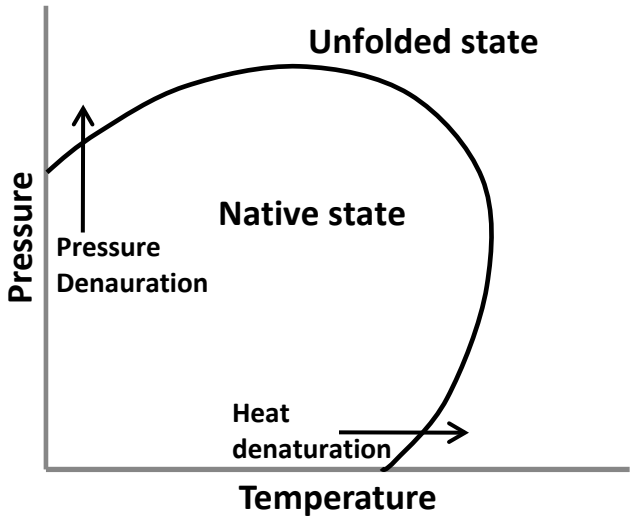

Figure 3: General schematic for the relationship of temperature and pressure to protein denaturation adapted from previous literature (Heremans, K. 2001).

The nature of HP-induced protein unfolding and denaturation is not fully understood. It has been proposed that HP protein unfolding is a multiple step process involving partially unfolded states that are reversible. As pressure is applied, intermediates are formed and play an important role in aggregation upon depressurization. The pressure induced conformations are more susceptible to aggregation as hydrogen bonds can form at lower temperatures under pressure (Heremans, K. 2001). Protein denaturation is generally reversible at lower pressures between 100-300 MPa, while at higher pressures denaturation is irreversible (Rastogi, N.

K. et al 2007). Pressure denaturation is also less extensive than thermal denaturation as hydrogen bonds stabilize an irreversible intermolecular network of the heat unfolded proteins (Heremans, K. 2001).

HPP is also a potential tool to enhance food safety while decreasing the use of preservatives and limiting detrimental effects on functional and nutritional value. There are many factors that contribute to variability in resistance of a microorganism to HPP. Some of these factors include the food system/medium, Gram +/-, and life cycle of the bacteria cell. In general, Gram positive bacteria are more resistant to HPP than Gram negative bacteria. Vegetative microorganisms in the growth phase are also more susceptible to HPP. Application of pressure in cycles has also been found to increase inactivation of microorganisms (Ponce, E. et al 1998a). HPP microbial reduction is normally caused by rupture of bacteria cell membranes (Ponce, E. et al 1998b). Although HPP is effective in reduction of many microorganisms, spores are resistant up to 1200 MPa (San Martin, M. F. et al 2002). There are several studies involving the effects of HPP on food pathogens in whole liquid egg (Ponce, E. et al 1998a; Ponce, E. et al 1998b, Ponce, E. et al 1999).

Egg pasteurization (60°C, 3.5 min) was designed to kill the most prevalent food pathogen in eggs and egg products, *Salmonella enteritidis*. *S. enteritidis* is of importance to food safety as it can cause severe gastroenteritis and is more heat resistant than other strains of *Salmonella*. The potential for HPP to supplement current thermal treatments of whole liquid for microbial inactivation of *S. enteritidis* has been investigated (Ponce, E et al 1999). *S. enteritidis* inoculated (10^7-10^8 CFU/mL) in whole liquid egg white was effectively destroyed via HPP. An 8 log reduction was achieved at 20°C at 450MPa/10

min with 5 minute cycles. Normally commercial eggs normally contain less than 10 *S. enteritidis* cells/egg. Thus, HPP in conjunction with mild heat treatment, is effective in destruction of *S. enteritidis* (Ponce, E. et al 1999)

Ponce, E. et al (1998a) investigated the effect of HPP on *Listeria innocua* as a model for *L. monocytogenes*, an important food pathogen. Liquid whole egg was inoculated with 10^6 CFU/mL and subjected to various levels of pressure and temperature. *L. innocua* was not fully inactivated by any of the treatments with the most effective treatment being over a 5 log reduction at 20°C at 450MPa/15 min with 5 minute cycles. Increasing pressure and time resulted in increased microbial inactivation. Temperature also played a large role as lower temperatures were more effective in *L. innocua* inactivation at lower pressures. In conclusion, HPP should be effective for levels (1 CFU/ml) of *L. monocytogenes* found in commercial eggs (Ponce, E. et al 1998a).

Another common food pathogen studied with HPP in whole liquid egg is *Escherichia coli* (Ponce, E. et al 1998b). Whole liquid egg was inoculated with 10^6-10^7 *E. coli* 405 and subjected to HPP at various temperatures. Pressure was the most important factor in inactivation followed by temperature and time. As pressure and time were increased, inactivation of *E. coli* was also increased. *E. coli* reduction was optimal at 50°C with 5 min cycles at 400MPa/15min and 450 MPa/10min with a 7 log reduction.

Raman Spectroscopy

Raman spectroscopy is a method that can offer structural information of egg white proteins before and after HP or heat treatment. The basis of Raman spectroscopy is the excitation of the ground electronic state of a molecule and the resulting vibrational transitions. The excitation and higher energy state is achieved by directing a

monochromatic laser or infrared light beam at a sample. As the molecule transitions back to a lower energy level a photon is scattered. The difference in frequency between the photon and the light source can be detected and is known as the Raman shift. The intensity of scattered light is plotted as a function of the change in wavenumber shift, giving the Raman spectrum. Changes in the Raman shift of peaks (vibrational frequencies) and their intensities correspond to changes in protein chemical structures and functional groups (Herrero, A. 2008). This provides information on the changes in secondary structure of food systems. Secondary structure of proteins has various motifs including α-helix, β-sheet, turns, and random coils.

Due to the fact Raman spectroscopy gives a very weak scattering signal of water, it is advantageous in the study of food proteins. This is important as many common food matrices contain more than 75% water (Beattie, R. et al 2004). Recently, vibrational spectroscopy has been used to analyze various food proteins including milk, beef, and fish (Li-Chan E. et al 2007; Beattie, R. et al 2004; Badii, F. et al 2006). Studies have also focused on the effects of HP and heat treatment on egg white proteins like ovalbumin. In HP and heat treated egg white albumen, changes in the amide III region indicate change in β-sheet structure with less β-sheet formation in HP samples (Ngarize, S. et al 2004b). There is also less of an effect on disulfide bonds using HP (400-600 MPa) when compared to heat (Ngarize, S. et al 2005). Other studies show that heating pure ovalbumin resulted in an increase of β-sheet with a loss of α-helix secondary structure. HP samples doubled the amount of β-turns, leaving the β-sheet/α-helix ratio relatively the same. Denaturation of ovalbumin was also shown to be less extensive at 600 MPa for 20 minutes compared to heat at 90°C for 30 minutes (Ngarize, S. et al 2004a). Irreversible

changes in the secondary structure of ovalbumin have also been reported at pressures over 400 MPa with a reduction in α-helix content and increase in β-sheet using circular dichroism and Fourier transform infrared spectroscopy (Smith, D. et al 2000). Another study using CD-spectroscopy showed that additions of NaCl to ovalbumin solutions reduced the loss of secondary structure (Iametti, S. et al 1998).

Protein Digestibility

Protein digestion in-vivo involves the secretion of several digestive enzymes. When protein is ingested, hydrochloric acid is secreted in the stomach, followed by the release of the first major digestive enzyme, pepsin. Pepsin preferentially cleaves at hydrophobic amino acid residues Phe, Tyr, Trp and Leu and is most active below pH 2. Hormones promote secretion of sodium bicarbonate into the small intestine, raising the pH to 8. The proteolytic enzyme trypsin (active at pH 8) is released and cleaves on the carboxyl side of Lys and Arg, except when followed by Pro. Trypsin facilitates the release of another major digestive enzyme, chymotrypsin, which cleaves on the carboxyl side of Tyr, Try, and Phe. Both trypsin and chymotrypsin cleave proteins with much greater specificity than pepsin. Other digestive enzymes include procarboxypeptidases and proelastase (Lehninger, A. et al 2005).

The in-vitro enzymatic digestion of egg white proteins has been subject to previous investigations. Mine Y. et al (2004) investigated the enzymatic digestion of egg white lysozyme. Egg white lysozyme hydrolysate was digested first by pepsin then trypsin. This approach was taken as lysozyme is generally resistant to trypsin alone but not to pepsin. It was found that pre-digestion by pepsin significantly increased hydrolysis by trypsin. There was no significant difference between digests of native and heat-

denatured lysozyme using a combination of pepsin and trypsin when analyzed with SDS-PAGE. Another group studied pepsin digestion of egg white ovomucoid (Kovacs-Nolan J. et al 2000). Large fragments of ovomucoid were found after 6 hours of peptic digestion. Unlike lysozyme, ovomucoid retained its resistance to trypsin after peptic digestion at enzyme to protein ratios of 1:20 and 1:200 (pH 2).

In-vitro digestion of ovalbumin that is similar to physiological conditions has also been investigated (Martos, G. et al 2010). At pH of 2 or above ovalbumin is very resistant to pepsin digestion at a 1:20 (enzyme: protein) ratio. However, the presence of bile salts increases digestibility. The most effective peptic digestion was achieved at pH 1.2 with a 3:1 (enzyme: protein) ratio. The study also indicated that pH, not enzyme ratio, is the more important factor in the peptic digestion of ovalbumin. HP-treatment on 10% egg white solutions has been shown to increase hydrolysis by trypsin (Iametti, S. et al 1999). Hydrolysis by α-chymotrypsin also increases with HP-treatment (Van der Plancken, I. et al 2004). Quiros and others (2007) added pepsin, trypsin, and chymotrypsin before HP-treatment on ovalbumin and found that this combination facilitated the release of peptides and increased ovalbumin susceptibility to enzymatic attack.

Egg white is an important source of dietary protein. This is primarily due to the high bioavailability of egg white protein and high content of essential amino acids. Two major factors affect the digestibility of egg white protein; digestive health and the components in food. The bioavailability of egg protein increases from 65% in raw egg to 95% in cooked egg protein (Seuss-Baum, I. 2007). Pressure induced egg white gels are more digestible than boiled egg white. HPP treatment also does not destroy vitamins or

amino acids (Hayashi, et al 1989). This indicates a greater bioavailability of protein and vitamins leading to increased nutritional value. Another aspect of nutrition to consider is the initiation of harmful chemical reactions that can occur during processing. Heat treated egg white produced lysinoalanine, an amino acid known to be a renal toxic factor in rats (Sternberg, M. et al 1975), while this compound was not detected in HP induced-gels (Hayashi, et al 1989). In addition to simple nutrition, processing can also facilitate the release of bioactive peptides (Kovacs-Nolan, J. et al 2005). Bioactive peptides isolated from egg white protein sources and their potential health benefits are discussed in a later section.

Allergenicity

An allergy is an immune initiated response mediated by immunoglobulin (Ig) E, causing a state of hypersensitivity (Kovacs-Nolan, J. et al 2000). An allergenic reaction is caused by specific food proteins with the ability to cross the intestinal barrier and causing an immune response. This involves binding of allergenic epitopes to IgE antibodies, releasing histamine receptors that trigger an inflammatory immune response or allergic reaction. The most common food allergies are IgE mediated (Mine, Y. et al 2008). The eight major food allergens include milk, eggs, fish, crustacean shellfish, tree nuts, peanuts, wheat, and soybean (FDA.gov, 2010). Common characteristics of food allergens include water solubility, heat and acid stability, and resistance to proteolytic digestion (Mine, Y. et al 2002). Food allergens must also be able to cross the intestinal barrier and still be large enough to bridge to IgE receptors on mast cells (Kovacs-Nolan, J. et al 2000; Mine, Y. et al 2008).

Allergenic reactions to eggs are mostly associated with egg white proteins. Common egg allergenic proteins include ovomucoid, ovalbumin, lysozyme, and ovotransferrin. Ovomucoid is considered the most dominant egg allergen (Mine, Y. et al 2006). Ovalbumin has many of the properties of an allergenic protein as it is resistant to enzymatic digestion, is water soluble, and is stable during processing (Mine, Y. et al 2002). The most common allergenic reaction to ovalbumin is a type 1 reaction involving IgE-binding to anti-ovalbumin antibodies of a patient sera. Most ovalbumin allergenic epitopes are 6-12 amino acids in length and recognition by anti-ovalbumin antibodies is sequential (Mine, Y. et al 2006). Ovalbumin allergenic epitopes are mostly composed of hydrophobic amino acids located within β-sheet and β-turn secondary structures. One major epitope is composed of a single α-helix (Mine, Y. et al 2003).

Processing conditions may also have an effect on the potential allergenicity of ovalbumin. Lopez-Exposito, I. et al (2008) studied the effects of HP at 400MPa with pepsin on the proteolysis profile of ovalbumin and its effects on IgG and IgE binding. Their results show an increase in proteolysis and less reactivity to IgG and IgE binding, suggesting a possible reduction in allergenicity of ovalbumin due to HP treatment. Other groups have found that heat also decreases enzymatic resistance of ovalbumin, but IgE binding was still present in soft and hard boiled eggs (Mine, Y. et al 2008). Kovacs-Nolan, J. et al (2000) also reported a reduction in IgE binding enzyme digested fragments of another egg white protein, ovomucoid. Irreversible changes in the secondary structure of ovalbumin have also been reported at pressures over 400 MPa with a reduction in α-helix content and increase in β-sheet using CD and FTIR spectroscopy (Smith, D. et al

2000). The loss of secondary structure may have an effect on ovalbumin allergenicity based on the structural location of sequential epitopes.

Various forms of processing have the potential to alter or reduce egg white allergenicity as discussed earlier. These methods include heat treatment, enzymatic fragmentation, and non-thermal techniques like HPP. Since most food allergenic epitopes are thought to be sequential, these processes are aimed at breaking apart epitopes and reducing allergenicity by increasing protein digestibility (Mine, Y. et al 2008). Potential reduction of food allergens are mostly reported based on the reduction of IgE and IgG binding response to specific epitopes (Lopez-Exposito, et al 2008; Kovacs-Nolan, J. et al 2000), which is necessary to produce an allergic reaction. The goal of industry is to use these food processing techniques to produce hypoallergenic products. However, the most prevalent way to avoid egg allergy is by strict avoidance of egg containing products (Mine, Y. et al 2008).

Bioactive Peptides

Bioactive peptide sequences are embedded within a protein and become active when released. In foods they are usually released via enzymatic hydrolysis and may have an influence on health. Bioactive peptides can also be released by proteolytic microorganisms and plant proteolytic enzymes (Korhonen, H. et al 2006; Minkiewicz, P. et al 2008). In addition, protein denaturation by food processing like thermal or HPP can facilitate the release of bioactive peptides. A large range of bioactive peptides have been isolated from food sources including opioid, immunodulatory, antimicrobial, mineral binding, growth and muscle stimulating, protease, antioxidant, and angiotensin-

converting enzyme ACE inhibitor peptides (Korhonen, H. et al 2006; Murray, B. A. et al 2007).

By far, milk is the most important and widely studied food system associated with bioactive peptides. Many bioactive peptides have been discovered and identified in milk with a wide range of functionality (**Figure 4**). The two primary methods for production of these include enzymatic hydrolysis and fermentation by lactic acid bacteria (LAB). Proteolytic enzymes have also been isolated from LAB for use in production of milk-derived bioactive peptides. As a result of LAB use in fermented dairy products, there is potential for increased concentration and possible health benefits. In fact, bioactive peptides have been isolated from finished fermented dairy products such as cheese, yogurt, and fermented milks.

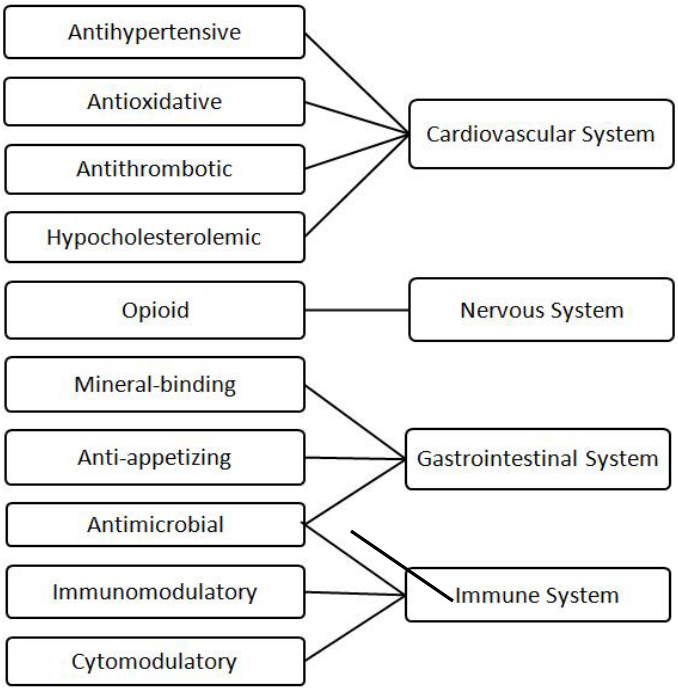

Figure 4: Milk-derived bioactive peptide functions (adapted from: Korhonen, H. et al 2006)

Bioactive peptides must also be able to exert a physiological effect once consumed. For example, milk-derived bioactive peptides shown to have potent ACE-inhibitory activity in vitro failed to have any significant ACE inhibition in rats (Fuglsang, A. D. et al 2003). One conclusion made was in order for a peptide to be physiologically active it must remain intact during digestion and be absorbed into the blood stream (Korhonen, H. et al 2006). The most well-known ACE-inhibitory milk peptides include tripeptides, VPP and IPP with IC_{50} values of 9.13 ± 0.21 and 5.15 ± 0.17 μM, respectively (Pan, D. et al 2004). IPP and VPP have been isolated via enzymatic digestion of β-casein (Shahidi, F. et al 2008). In human studies, IPP and VPP, administered in sour milk (2.6 mg peptide/day) to hypertensive individuals resulted in a reduction of blood pressure (Hata, Y. et al 1996). Other ACE-inhibitory milk peptides have shown in-vivo activity in humans and rats and have been subject to extensive review (Korhonen, H. et al 2006, Shahidi, F. et al 2008). The investigations of bioactive peptide production from milk and the demonstrated health benefits have resulted in research in other food systems, including egg.

Many bioactive peptides derived from egg white proteins via enzymatic hydrolysis have been identified. These peptides mostly have ACE-inhibitory and antihypertensive effects. Novel antihypertensive peptides derived from egg white proteins have been shown to have blood pressure lowering effects on spontaneously hypertensive rats (SHR) (Miguel, M. et al 2007a). Enzymatic hydrolysis of ovalbumin has been shown to release ACE-inhibitory peptides (Quiros, A. et al 2007; Miguel M. et al 2006a; Miguel M. et al 2007b). One study isolated ovokinin (FRADHPFL) and ovokinin 2-7 (RADHPF) from ovalbumin, in which both showed antihypertensive effects

on SHR rats (Miguel, M. et al 2006b). A recent study isolated an ACE inhibitor peptide (RVPSL) from egg white protein ovotransferrin (Liu, J. et al 2010). Antimicrobial peptides (IVSDGDGMNAW and HGLDNNYR) have been isolated from egg white lysozyme hydrolysate via hydrolysis by pepsin and trypsin. These water soluble peptides exhibited bacteriostatic activity against E. Coli K-12 and S. aureus, respectively. Incubation with target bacteria of each peptide at a concentration of 400 µg/mL resulted in damage to cell membranes through direct interaction (Mine, Y. et al 2004). Table 2 summarizes some of the bioactive peptides isolated from egg proteins.

Table 2: Bioactive peptides isolated from egg proteins with antihypertensive activity (Miguel, M. et al 2006b)

Sequence	**Origin**	**Enzyme**	**Bioactivity**
FRADHPFL	Ovalbumin	Pepsin	Vasorelaxing/Antihypertensive
RADHPF	Ovalbumin	Chymotrypsin	Vasorelaxing/Antihypertensive
RADHPFL	Egg white	Pepsin	ACE-inhibitor/Antihypertensive
YAEERYPIL	Egg white	Pepsin	ACE-inhibitor/Antihypertensive
IVF	Ovalbumin	Pepsin	ACE-inhibitor
FGRCVSP	Ovalbumin	Pepsin	ACE-inhibitor
ERKIKVYL	Ovalbumin	Pepsin	ACE-inhibitor
FFGRCVSP	Ovalbumin	Pepsin	ACE-inhibitor
LW	Ovalbumin	Pepsin	ACE-inhibitor/Antihypertensive
FCF	Ovalbumin	Pepsin	ACE-inhibitor
NIFYCP	Ovalbumin	Pepsin	ACE-inhibitor
RADHP	Egg white	Pepsin/Corolase PP	ACE-inhibitor/Antihypertensive
Oligopeptides	Egg yolk	Several enzymes	ACE-inhibitor/Antihypertensive

The effectiveness of a compound to inhibit a biological activity is measured by the half maximal inhibitory concentration (IC_{50}). This concentration corresponds to the amount of compound needed to inhibit a particular biological function by 50%. The most effective ACE-inhibitor peptides corresponded to the sequences FFGRCVSP, ERKIKVYL, and FRADHPFL with IC_{50} values of 0.4, 1.2, and 3.2 µM respectively (Miguel, M. et al 2006a; Fujita, H. et al 2000). The peptide with the sequence FRADHPFL, known as ovokinin, has been found to exert vasorelaxing effects in canine

mesenteric arteries and lower systolic blood pressure in SHR rats. Hydrolysis of ovokinin results in the formation of peptides with the sequences of RADHPF and RADHP. These peptides are much weaker ACE-inhibitors with IC_{50} values of 514 and 257 µM respectively. In addition to ACE-inhibitory properties, the sequences YAEERYPIL and FRADHPFL exhibit radical scavenging activity with an oxygen radical absorbance capacity fluorescein assay (ORAC-FL) value of 3.8 and 0.128 µmol of Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) equivalent/µmol of peptide (Miguel, M. et al 2006a). Table 3 on the next page gives each peptide and their ACE-inhibitory IC_{50} value.

Table 3: Bioactive peptides derived from egg white with ACE-inhibitory IC_{50} values (Miguel, M. et al 2006a; Miguel, M. et al 2007a; Fujita, H. et al 2000; Liu, J. et al 2010)

Peptide Sequence	**IC_{50} (µM)**	**Residue Sequence in Ovalbumin**
FRADHPFL	3.2	358-365
RADHPF	514	359-364
RADHPFL	6.2	359-365
YAEERYPIL	4.7	107-115
IVF	33.1	178-180
FGRCVSP	6.2	379-385
ERKIKVYL	1.2	274-282
FFGRCVSP	0.4	378-385
LW	6.8	183, 184
FCF	11	10-12
NIFYCP	15	26-31
RADHP	257	359-363
		Residue Sequence in Ovotransferrin
RVPSL	20	328-332

ExPASy Peptide Cutter was used to project theoretical peptides derived from ovalbumin using pepsin at pH 1.3. The full ovalbumin sequence given shows the cleavage sites predicted by Peptide Cutter (**Figure 5**). A table with a list of the sequences and pepsin cleavage sites is given in the appendix.

1 MGSIGAASME|F|C|F|DV|F|KE|LK VHHANENI|F|Y CPIAIMSA|L|A MV|Y|L|GAKDST RTQINKVVRF|
61 DKL|PGF|GDSI EAQCGTSVNV HSSL|RDIL|NQ ITKPNDV|Y|S|F| S|L|ASRL|YAEE RY|PIL|PEY|L|Q
121 CVKE|LY|RGGL EPIN|F|QTAAD QARE|LINS|W|V ESQTNGIIRN VLQPSSVDSQ TAMV|L|VNAIV|
181 F|KG|LW|EKT|FK DEDTQAMP|FR VTEQESKPVQ MM|Y|QIG|L|F|RV ASMASEKMKI|LEL|P|FASGTM
241 SM|L|V|LL|PDEV SG|L|EQ|L|ESII N|F|EK|L|TE|W|TS SNVMEERKIK V|YL|PRMKMEEK|Y|NL|TSV|L|MA
301 MGITDV|F|SSS AN|L|SGISSAE S|L|KISQAVHA AHAEINEAGR EVVGSAEAGV DAASVSEE|F|R
361 ADHP|FL|F|CIK HIATNAV|L|F|F| GRCVSP

Figure 5: Ovalbumin sequence (NCBI Reference Sequence NP_990483.1, 2010) with cleavage site predicted by ExPASy Peptide Cutter with pepsin at pH 1.3. Cleavage sites are represented by single vertical lines. The highlighted sequences are those that have already been identified.

Other potential bioactive peptides in egg white proteins have been identified via sequence alignment. Milk-derived ACE inhibitor IPP is embedded in α-ovomucin (1365-1367) and ovotransferrin (527-529). Milk-derived ACE inhibitor VPP is also sequenced in α and β subunits of ovomucin (α-1647-1649, β-765-766). ACE inhibitory peptide YP, derived from whey protein, is found in the sequence of ovalbumin (112-113) and ovomucoid (163-164).

Egg White Functional Properties

Gelation

Gelation in egg white protein begins with the native proteins being unfolded or denatured. Spherical aggregates form due to hydrophobic interactions, making the solution turbid. The aggregates thicken by stabilization via sulfhydryl-disulfide reactions (ovalbumin). This is followed by coagulation and gelation as a result of the rapid re-formation of hydrogen bonds (Mine, Y. et al 1995). Ovotransferrin also plays an important role in gelation as it is the first egg white protein to thermally denature and initiate coagulation (Croguennec, T. et al 2002). The formation of egg white gels can be induced by either heat, HP treatment, or under acidic conditions.

Egg white protein gelation is influenced by multiple factors including temperature, pressure, pH, and salt concentration (ionic strength). Various forms of egg gels can form under thermal denaturation depending on the pH and ionic strength. When the pH is near the pI of the proteins in solution (most egg white proteins are the pI ranges from 4-5) or the ionic strength is high denatured proteins aggregate randomly via hydrophobic interactions (Nakamura, R. et al 2000). As the pH nears the pI of the proteins the net charge on the proteins is reduced resulting in an increase in hydrophobic interactions, followed by aggregation. An increase in salt concentration, independent of pH, also decreases repulsive forces between proteins as negative charges are shielded by Na^{+2} ions leading to increased hydrophobic protein-protein interactions (Croguennec, T. et al 2002). These conditions produce an opaque and turbid gel (Nakamura, R. et al 2000) as the aggregates tend to be coarse and large (Croguennec, T. et al 2002). Thus, the gel network is "loose" as it is composed of large aggregates bound together via hydrogen bonds and disulfide interactions, reducing water holding capacity (WHC) (Barbut, S. 1996). One study (Croguennec, T. et al 2002) reported a >15% weight loss of a heat-induced egg white gel formed at pH 5 due to syneresis. Protein gels heated above 80°C are also more prone to syneresis and shrinkage (Nakamura, R. et al 2000).

In contrast, when the pH is further from the pI (above or below) or there is low ionic strength the denatured proteins tend to aggregate in an ordered linear manner forming a more transparent gel. This is due to a decrease in electrostatic interaction at lower ionic strength (Nakamura, R. et al 2000). The aggregates are also smaller, forming a tighter gel network with increased WHC (Barbut, S. 1996). Croguennec, T. et al (2002) indicated that pH was the most important factor for the viscoelastic properties

of egg gels. Their group found that the natural rise in pH of egg during storage resulted in gels with increased elasticity, penetration force, and viscosity index. This increase in gel strength may be due to increased disulfide exchange after gel formation. Lowering the pH resulted in weaker gels. NaCl was also found to increase gel strength at pH 5, but had little effect at pH 7 and 9 (Croguennec, T. et al 2002). One study involving the production of Chinese thousand year old eggs showed that the formation and properties of egg gels are also highly dependent on pH and salt concentration (Eiser, E. et al 2009). Another factor that influences egg white gel formation is protein concentration as this affects the formation of insoluble aggregates (Iametti, S. et al 1998). At low concentrations a more translucent gel is formed while higher concentrations produce more opaque, turbid gels (Doi, E, et al 1997). A summary of gel properties with under various conditions is illustrated in the **Figure 6.**

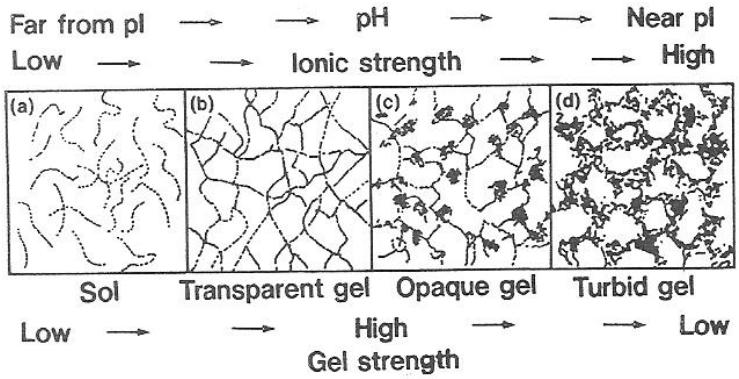

Figure 6: Illustration of gel properties due to changes in pH and ionic strength (Doi, E, et al 1997).

Another method to produce food gels is under cold acidic conditions. This is a 2 step process in which the proteins are first denatured thermally at neutral pH and low ionic strength forming soluble aggregates in solution. Gelation is then induced by lowering the pH to the isoelectric point of the proteins, reducing electrostatic interactions

and increasing aggregation/gelation (Weijers, M. et al 2006). Weijers, M. et al (2006) investigated the production of transparent egg white gels using egg white powder. Removal of ovotransferrin was required to induce transparent gel formation as it interferes with fibril/ordered formation of transparent gel networks. This was explained by disulfide interactions between ovotransferrin and ovalbumin resulting in clusters of aggregates and hindering linear gel formation (Weijers, M. et al 2006). This also explains why egg white usually forms opaque gels (Nakamura, R. et al 2000). During acid-induced gel formation, fewer disulfide bonds are formed between protein molecules. The disulfide bonds are instead formed after the gel is cold-set (Weijers, M. et al 2006). This supports the conclusion found by Broersenn, K. et al (2006) that disulfide interactions are not the driving force of ovalbumin aggregation, but rather stabilize the gel network after it is formed.

The coagulation of egg white by pressure was first observed by Bridgman (1914). Both heat and HP treatments on ovalbumin have been shown to expose hidden –SH groups via the Ellman's reagent method (5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB)). These exposed groups stabilize protein aggregates, which lead to gelation (Van der Planken, I. et al 2005b, 2007b). Ngarize and others (2005) reported that pressure induced gels were more glossy and smooth in appearance than heat treated gels. HP-induced egg white gels are generally softer and more elastic than heat induced ones (Hayashi, R. et al 1989; Ngarize, S. et al 2005). HP gels were rubbery compared to heat treated gels, which were more hard and brittle. These results were based on egg white treated between 400-600 MPa and heat induced gels formed via 90°C for 30 min. Gel strength values were comparable to heat induced gels at pressures over 650 MPa. Egg white showed no

gelation at pressures of 500 MPa for 20 min (Ngarize, S. et al 2005). Prevention of gel formation due to HP (800 MPa, 10 min) has been accomplished by adding NaCl or sucrose to 10% egg white solutions prior to HP treatment (Iametti, S. et al 1999). The taste and flavor of HPP egg white gels is natural and uncooked (Hayashi, R. et al 1989).

Foaming

The major egg white proteins that are important to foaming are ovalbumin, ovomucin, ovotransferrin, lysozyme, and globulin proteins (Mine, Y. et al 1995). Ovalbumin plays a central role in egg white foaming abilities. When whipped, ovalbumin molecules are adsorbed in the air/water interface and the hydrophobic areas of the protein are oriented towards the gas phase of the interface. The conformational changes in structure expose buried sulfhydryl groups which then become oxidized. This results in the formation of disulfide bridges with adjacent ovalbumin molecules. Aggregates are then formed at the air/water interface and produce a gel network that provides stability for the foam. The strength of the foam network is derived from the non-covalent bonding and disulfide bridges formed between ovalbumin molecules as a result of its denaturation and conformational changes (Doi, E. et al 1997). Orientation of the hydrophobic areas towards the gas phase and hydrophobic interactions also provide stability for the film formed around the air pocket (Nakamura, R. et al 2000). Being the only egg white protein with free sulfhydryl groups, foams produced with ovalbumin tend to be more stable due to disulfide linkages (Lechevalier, V. et al 2003).

One of the main factors affecting foam formation is the ability of a protein to be adsorbed into the air/water interface and undergo rapid conformational change (Mine, Y. et al 1995). The structural modifications and conformational changes of ovalbumin,

ovotransferrin, and lysozyme at the air/water interface have been studied (Lechevalier, V. et al 2003; Lechevalier, V. et al 2005). Both ovalbumin and ovotransferrin undergo changes in secondary structure via foaming with a shift from α-helix to β-sheet structures (sheets and turns). This is especially evident with ovotransferrin as there is a 33% relative loss of α-helix and a 94% relative gain in β-sheet structure. Ovotransferrin has also been shown to increase in surface hydrophobicity, which is important to foam stability. In contrast, lysozyme does not undergo conformational change at the air/water interface, leading to poor individual foaming properties (Lechevalier, V. et al 2003). However, lysozyme contributes to the "synergy" of the protein mixture in egg white during foaming as it is unfolded and involved in electrostatic interactions with other proteins (Lechevalier, V. et al 2005). The electrostatic interactions between proteins contribute to the foaming ability of egg white and its heat stability characteristics (Mine, Y. et al 1995). Ovotransferrin contributes to this synergy via covalent aggregates at the air/water surface as it is the most denatured protein during foaming (Lechevalier, V. et al 2003; Lechevalier, V. et al 2005).

Two of the most common measures of foaming properties of egg white include foam overrun and foam stability. Foam overrun (OR) is defined as the foam volume measured against the initial liquid volume of the solution before foaming. Foam stability (FS) is a measured by the amount of liquid drainage from the foam in relation to the initial liquid volume before foaming. The following equations define these measurements where Vf is foam volume, Vl_i is initial liquid volume, and DV is drained volume (Lomakina, K. et al 2006).

$$\text{Foam Overrun} = Vf/Vl_i$$

$$\text{Foam Stability} = ((Vl_i - DV)/Vl_i) \times 100\%$$

There are many factors that affect egg foam properties including but not limited to salt concentration, sugar content, pH, and processing conditions. These factors have been extensively reviewed due to egg white's importance as a functional food ingredient (Lomakina, K. et al 2006). Addition of NaCl enhances foaming ability and increases foam overrun. The salt reduces protein-protein interactions (electrostatic repulsion) allowing them to unfold more readily and be incorporated in the air/water interface, thus increasing foaming capacity. Addition of sugar to egg white often decreases foam expansion but increases foam stability due to an increase in viscosity (Raikos, V. et al 2007). The increased stability is achieved by the sugar binding excess water while the reduced expansion can be explained by the sugar's stabilizing effects on protein structure (increase protein-protein interactions). Ovomucin may contribute to foam stability in this manner due to its long carbohydrate chains that can retain water (Hammershoj, M. et al 2008).

With respect to pH foam overrun is highest at pH 4.8 and lowest at 10.7. There is also an increase in foam overrun as pH naturally rises in egg white over time. However, foam stability of aged egg white decreases due to an increased concentration of s-ovalbumin (less hydrophilic) in old eggs and its interference with film formation around the air bubble. The stability of egg white is highest at egg white natural pH of 8.6 (Lomakina, K. et al 2006). Lysozyme is positively charged at this pH and has the ability to interact with negatively charged proteins via electrostatic interactions (Mine, Y. et al 1995).

Generally whipping time increases foaming ability, although excess whipping can reduce foam stability as smaller bubbles are formed. Yolk contamination decreases foaming ability as components of yolk can complex with ovomucin hindering foam formation. Pasteurization decreases foaming abilities of egg white due to the formation of an ovomucin-lysozyme complex when ovotransferrin is denatured at 53°C. Removal of this complex is necessary to regain normal foaming properties (Lomakina, K. et al 2006). Additions of metallic ions like Cu^{+2} that can bind and stabilize ovotransferrin are used to retain foaming properties of pasteurized egg white products (Nakamura, N. et al 2000). Heat is used to "set" egg white foams via coagulation that produces a stable structure (meringues) (Mine, Y. et al 1995).

HP treatment has been shown to have positive effects on the functional properties of egg white. First, HP treated egg albumen retains its foaming and heat induced gelation properties (Iametti, S. et al 1999). While both HP and heat affect the foaming properties of egg white, HPP has been shown to increase its foaming abilities. Van der Plancken and others (2007a) studied the foaming properties of 10% egg white solutions at pH levels corresponding to fresh (7.6) and aged egg white (8.8). The best foam with respect to most volume and average density has been reported with HP treated egg white at pH 8.8. However, the highest density foam was reported with non-treated egg white at pH 7.6. Both HP and heat treatments were shown to reduce collapse in foamed egg white.

Purpose of Work

Egg white proteins are an important and desirable ingredient to the food industry due to their functional properties which include gelling, foaming, emulsification, and binding adhesion. These properties are incorporated in many products like meringues, processed meat products, and baked goods (Mine, Y. et al 1995). As a result of egg white being a valuable ingredient to the food industry, it is important to determine the effects of processing on egg white proteins. HPP is an alternative non-thermal food processing method that has shown promise in the development of new food products with added functional and health benefits. Thus, the purpose of this research was to evaluate the effects of HPP on egg white protein and the impact it has on egg white protein digestibility and egg white as a functional food ingredient. Egg white is well recognized as an excellent nutrition source and this work is aimed at increasing the understanding of its potential health benefits in terms of bioactivity and allergen reduction.

Materials and Methods

High Pressure Treatment

Eggs were obtained from a local supermarket and the egg white was separated from the yolk. Eggs were grade A large and had a pH of 9.1. Samples of egg white were placed in sausage casing and vacuum sealed in polyethylene bags for HP treatment. Pressure treatments were applied at 400, 600, and 800 MPa for 5 min at 4°C using a Stansted ISO-Lab High Pressure Food Processor. The temperature of the pressurization vessel was monitored and ranged between 4 and 10°C during processing. The processing fluid consisted of a propylene glycol/water mixture. Treated samples were stored at 4°C until analysis. HPP was repeated for all analyses to ensure consistent treatment conditions and results.

Raman Spectroscopy

Egg white was heated at 65, 85, and 95°C for 5 minutes and refrigerated overnight then placed in VWR glass vials (#66011-020). HP treated samples of egg white were prepared as described before (400, 600, and 800 MPa) and prepared for Raman analysis the same as the heat treated samples. Egg white without heat or HP treatment was analyzed as the control. Raman spectra were recorded at room temperature (~20°C) using an Enwave Optronics spectrometer. The laser excitation wavelength was 785 nm. Spectra were collected using an integration time of 120 s, with the averaging of 3 spectra, and boxcar smoothing set to 2. The spectra was analyzed for changes in protein secondary structure based on shifts and magnitude of peaks corresponding to the amide I and III regions.

In-vitro pepsin digestion

The in-vitro pepsin digestion protocol used was similar to the one described by Zeece et al. (2008). A stock pepsin (Sigma P6887) solution was prepared by dissolving 18 mg in 10 ml cold simulated gastric fluid (SGF) containing 0.1 N HCl, 0.03 M NaCl, pH 1.2 (Sigma G3285). The enzyme was completely dissolved by vortexing and placed on ice. The pepsin solution was used for a maximum of 2 hours for digestions, and then a fresh solution was prepared for subsequent digestions.

The HP and heat treated samples (85 and 95°C for 5 min) were diluted 1:10 in nanopure H_2O and homogenized with a brief 10 second pulse to uniformly distribute the sample in solution. Incubations were set up by adding 1.2 mL SGF-pepsin solution to a 1.5 mL microfuge tube. The incubation tubes were equilibrated in a 37°C water bath for 5 minutes. The digestion was initiated by adding 70 µL (~70µg egg protein) egg white sample. This gave an approximate enzyme to protein ratio of 3:1, assuming the whole egg white had a protein concentration of 10% (determined via BCA method using BSA as the standard). The digestion was stopped by withdrawing 200 µL from the incubation tube and placing it in a 1.5 mL microfuge tube containing solution **A**(80 µL Na_2CO_3 with 10 µL 10% SDS) at 30s, 2, 4, 8, 15, and 30 minutes. The samples were immediately vortexed and placed on ice. A control 0 time tube was prepared by adding 50 µg test protein to a tube containing 200 µL SGF-pepsin and solution **A**, which was vortexed and placed on ice. Control tubes were also prepared without SGF-pepsin by adding 50 µg test protein to 200 µL SGF with solution **A**. Additionally, a tube containing SGF-pepsin was incubated at 37°C for 30 minutes to monitor any pepsin self-digestion products.

Digestions with the control and 800 MPa sample were also completed with pepsin to protein ratio of 1:20.

SDS-PAGE

Each time point sample and controls were prepared by adding 35 µL tracking dye solution (Bio-Rad tricine sample buffer with β-mercaptoethanol) and heating at 50°C for 2 minutes. The samples were then centrifuged for 2 min at 10,000 g and stored at -20°C until SDS-PAGE analysis. SDS-PAGE was performed by loading 35 µL of sample (~13 µg protein) on 10-20% gradient tricine pre-cast Bio-Rad Criterion gels. Gels were stained overnight in 0.1% Coomassie Brilliant Blue R-250 with 50% methanol, 10% acetic acid and de-stained in 10% methanol, 7% acetic acid. Digital images of gels were taken and protein bands of interest were sent for MS analysis.

RP-HPLC

Samples were prepared for RP-HPLC analysis by performing the in-vitro pepsin digestion as before but using 90 µL 0.4 M NH_4HCO_3 as the stop solution. The digested samples were centrifuged for 10 min at 17,000 rcf and the supernatant was removed and filtered with a YM3 3000 molecular weight cut off filter (MWCO) spin filter at 14,000 rcf until 10% of original volume remained. The filtered samples were dried using a Centra-Vap and stored at -20°C until analysis. Dried samples were re-suspended in 50 µL 0.1% TFA in nanopure water and diluted for RP-HPLC analysis.

RP-HPLC of the digestion products was performed using a Waters 510 HPLC system equipped with a tunable absorbance detector set to 214 nm and a HP 3395 Integrator. The column was a 2.1×150 mm Waters XBridge BEH130 C_{18}; 3.5µm particle size. An automated gradient controller controlled the elution of solvent and the sample

was manually injected. The injection volume was 5 µL. Solvent A was 0.1% TFA in nanopure water and solvent B was 0.1% TFA in acetonitrile. The column was equilibrated with 100% A. The flow rate was 0.25 ml/min. Peptides were eluted with a linear gradient from 0 to 70% B in A over 15 min, then isocratic elution of 70% B, followed by 5 minute linear gradient to 100 % B. Chromatograms were compared to determine the profile of components and treatment induced changes.

Liquid Chromatography/Mass Spec/Mass Spce (LC/MS/MS) analysis

Samples were prepared for LC/MS/MS analysis by performing the in-vitro pepsin digestion as before but using 90 µL 0.4 M NH_4HCO_3 as the stop solution. The digested samples were centrifuged for 10 min at 17,000 rcf and the supernatant was removed and filtered with a YM3 3000 molecular weight cut off (MWCO) spin filter at 14,000 rcf until 10% of original volume remained. The filtered samples were dried using a Centra-Vap and stored at -20°C until purification. Samples were purified prior to MS analysis using Pierce PepClean^{TM} C-18 spin columns (#89873). Protocols for purification and clean-up were followed as described by the instruction manual. Eluted digests were dried using a Centra-Vap and stored at -20°C until being sent for LC/MS/MS analysis.

2-Dimensional (2D) Electrophoresis

Digestion samples of the control and 800 MPa at time 0 and 15m (1:20 pepsin to protein ratio) were subject to 2D analysis. The digestions were performed as described in RP-HPLC. An aliquot of digested sample containing approximately 200 ug protein was dried using a Centra-Vap and stored at -20°C until analysis. Each sample was rehydrated using 200 uL Bio-Rad Ready Prep 2D Rehydration/Sample Buffer (#1632106). First dimension separation of the proteins was performed using isoelectric focusing (IEF) with

Bio-Rad Ready Strips IPG with a pH range of 3-10 (#163-2000) following instructions from kit. After IEF was complete the strips were equilibrated for SDS-PAGE separation on a rocker tray for 5 minutes at room temperature in 5 mL buffer **I** (0.05 M Tris-Cl pH 6.8, 6M Urea, 1% SDS, 50 mM DTT), followed by buffer **II** (0.05 M Tris-Cl pH 6.8, pH 6.8, 6M Urea, 1% SDS, 50 mM iodoacetamide), and buffer **III** (0.05 Tris-Cl pH 6.8, 1% SDS).

SDS-PAGE was performed using 10-20% gradient Tris-HCl pre-cast Bio-Rad Criterion gels. Gels were stained overnight in 0.1% Coomassie Brilliant Blue R-250 with 50% methanol, 10% acetic acid and de-stained in 10% methanol, 7% acetic acid. Digital images of gels were taken and protein spots of interest were sent for MS analysis.

Texture and Color Analysis

The gel texture of HP treated egg white samples (600 and 800 MPa, 5 min) and the heat treated sample at 95°C for 10 min were analyzed. These were the only conditions analyzed that produced a gel suitable (non-runny, stable) for texture analysis. The pH of the egg white used for texture analysis was 9.1. The pH was also adjusted to 6.0 using tartaric acid to study the effects of lowered pH. Unlike previous HP treatments, samples for texture and color analysis were treated with a pressure vessel temperature set at 10°C with processing temperatures ranging between 30-40°C. Treatment for functional properties was completed on a 900 plunger press system (Standsted Fluid Power Ltd, Essex, UK) and accounts for the changes in treatment conditions. All other HPP conditions remained the same.

Textural properties were evaluated with a TA.XT2 Texture Analyzer with a 5 kg load cell. Egg samples were cut to a height of 20 mm with a diameter of 23 mm. The

sample was then compressed twice (2 bite cycle) with a 50% penetration value using a cylindrical probe (TA-4, 37 mm diameter). The compression speed was set to 1.2 mm/s. Texture Technologies texture profile analysis was used to determine the properties of each gel according to Bourne (1982). The properties analyzed were hardness, cohesiveness, springiness, gumminess, and resilience. Each sample was measured 5 times.

The color of the gels were analyzed using a Minolta colorimeter with the Hunter L, a, b color scale. Egg samples were cut to a height of 10 mm with a diameter of 23 mm. L, a, and b measurements were taken 5 times per sample using 10°/D65 as the light source.

Syneresis occurred in all gels analyzed. The discharged liquid was collected and subject to SDS-PAGE analysis. Conditions for electrophoresis were the same as previously described.

Foaming Ability

The foaming procedure used in this experiment followed a protocol similar to the one described by Van der Plancken et al (2007a). Egg white was diluted 1:10 in nanopure H_2O and stirred until homogenous (pH 9.1). Adjustment in pH of 10% solutions was achieved by using varying amounts of 0.02% potassium bitartrate (KT) in place of water to lower the pH to 6.0 and 4.5 prior to HP-treatment. The HP-treatment conditions were the same as those used in the texture and color analysis. Control samples at each pH were also analyzed.

A volume of 30 ml 10% egg white was placed in a 400 mL beaker and whipped using a motorized whisk for 2 minutes. The resulting foam and liquid was transferred

into a 250 mL graduated cylinder and air pockets removed with two quick downward shakes. The volume of the foam and liquid was recorded every 5 min for 15 min and at 30 min post-foam. Each sample was measured 5 times. Foam overrun and stability were calculated for each sample (Lomakina, K. et al 2006).

Statistical Analysis

Texture, color, and foaming properties were subject to analysis of variance to determine statistical significance ($p<0.05$) using SAS (Version 9.2, SAS Institute Inc., Cary, NC). Reproducibility of the results were analyzed using an F-test ($p<0.05$). Difference least squares means and Duncan's multiple range test were also used to determine statistical significance.

Results and Discussion

High pressure treatment has been used with a number of food systems (milk, muscle, soy, etc) to alter properties of constituent proteins and has been subject to review (Rastogi, N. K. et al 2007). Investigations of egg systems in particular, have shown that HP treatment denatures ovalbumin and alters foaming properties of diluted egg white (Smith, D. et al 2000, Van der Plancken, I. et al 2007a). Work presented here extends those investigations to gain more complete understanding of the effects of HP treatment on the complement of proteins found in egg white. To achieve this goal, Raman spectroscopy and in-vitro pepsin digestion were used to assess the effect of HP treatment on egg white proteins. Raman spectroscopy was used to determine protein conformational changes resulting from HP treatment. Similarly, in-vitro pepsin digestibility was used to determine the effects of HP treatment on the denaturation of egg white proteins and gain greater understanding of proteins affected by identifying the peptide products of digestion. The effect of HP treatment on egg white functionality (gelation, texture, foaming, and color) was also investigated.

Raman Spectroscopy

Raman spectroscopy is a method that offers structural information of egg white proteins before and after HP or heat treatment. This method has also been used to investigate structural changes in other food systems like milk, beef, and fish (Li-Chan E. et al 2007; Beattie, R. et al 2004; Badii, F. et al 2006). The regions of Raman spectra most useful in determining protein secondary structure are the amide I (1600-1700 cm^{-1}), amide II (1510-1560 cm^{-1}), and amide III (1200-1300 cm^{-1}) bands. Another important region for secondary structure is the C-C stretching region between 890-1060 cm^{-1}

(Herrero, A. 2008). The Raman spectrum of untreated control egg white is shown in **Figure 7**.

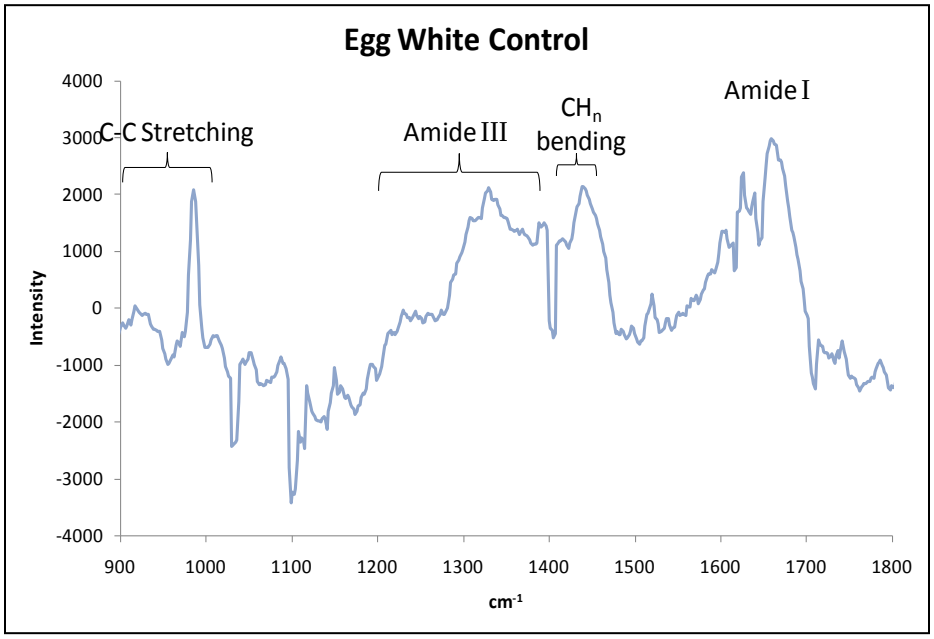

Figure 7: Raman spectra (900-1800 cm^{-1}) for untreated control whole egg white. Spectral regions important to protein secondary structure are labeled. The spectra were auto-baselined with Enwave Optronics software.

The control egg white spectra were used as a base for comparison to determine changes in secondary structure of HP and heated egg white (**Figure 8**) HP or heat treatment resulted in more pronounced and larger bands in the CH-stretching (1510-1560 cm^{-1}) and amide III (1200-1300 cm^{-1}) regions. This indicates an increase in hydrophobicity, most likely due to hydrophobic interactions involved in gelation and the formation of aggregates. Although not indicated in the amide III regions of the spectra, other studies examining the effects of HP and heat on ovalbumin found changes that indicated a decrease in β-sheet structure (Ngarize, S. et al 2004b).

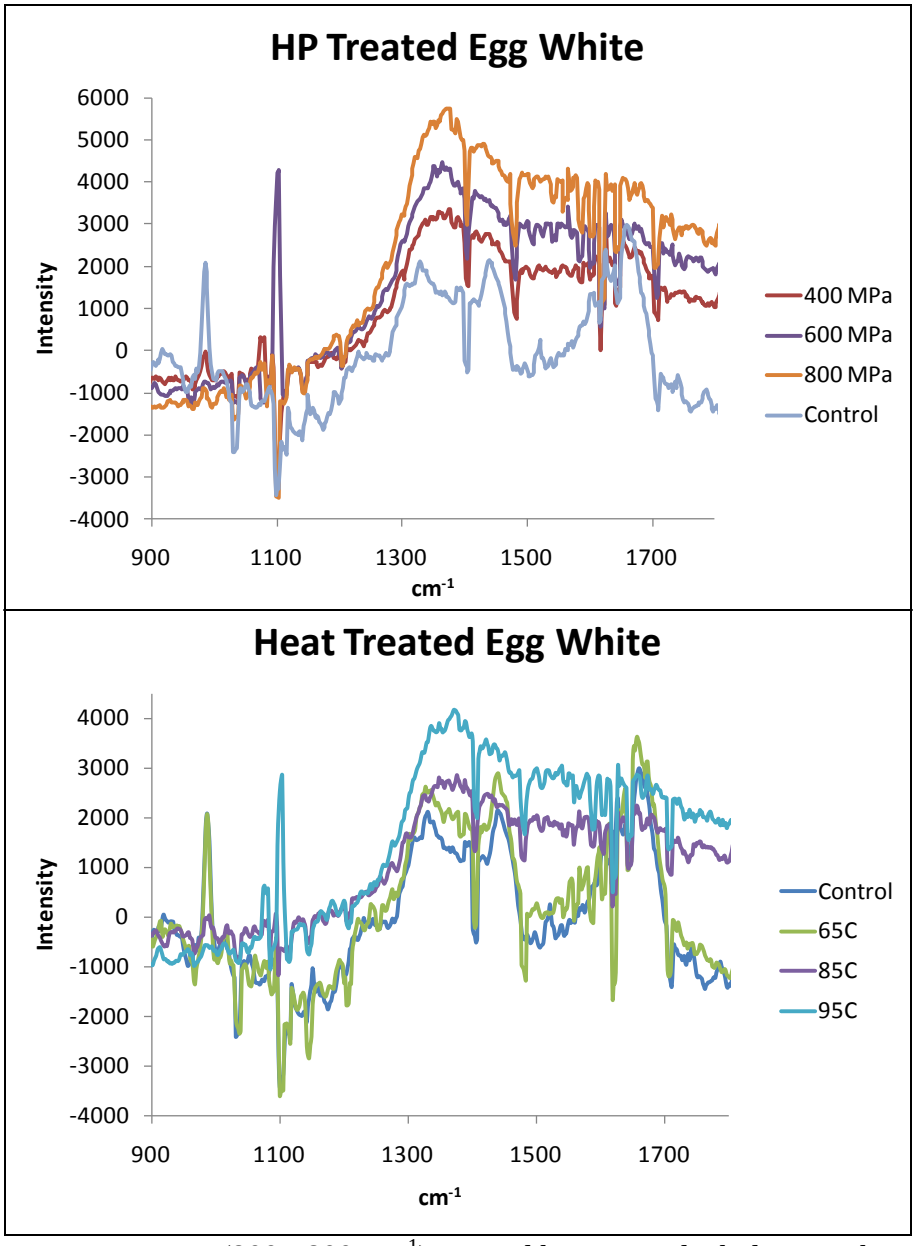

Figure 8: Raman spectra (900-1800 cm^{-1}) HP and heat treated whole egg white. The spectra were auto-baselined with Enwave Optronics software. See methods for measurement and treatment conditions.

There were several major changes observed in spectra between the control and treated samples with respect to protein secondary structure. A decrease in the band at 980-990 cm^{-1} was observed in HP-treated egg white and at temperatures 85°C and 95°C (**Figure 9**). This region of Raman spectra represents C-C stretching and corresponds to alpha helical content. Thus, HP and heat both contribute to a decrease in α-helical

structure upon treatment. Heat treatment at 65°C showed no apparent affect on secondary structure in this region of spectra. Previous comparisons between heat and HP-treated ovalbumin indicated protein denaturation was less extensive with pressure than heat. However, both altered secondary structure by increasing the β-sheet/α-helix ratio (Ngarize, S. et al 2004a).

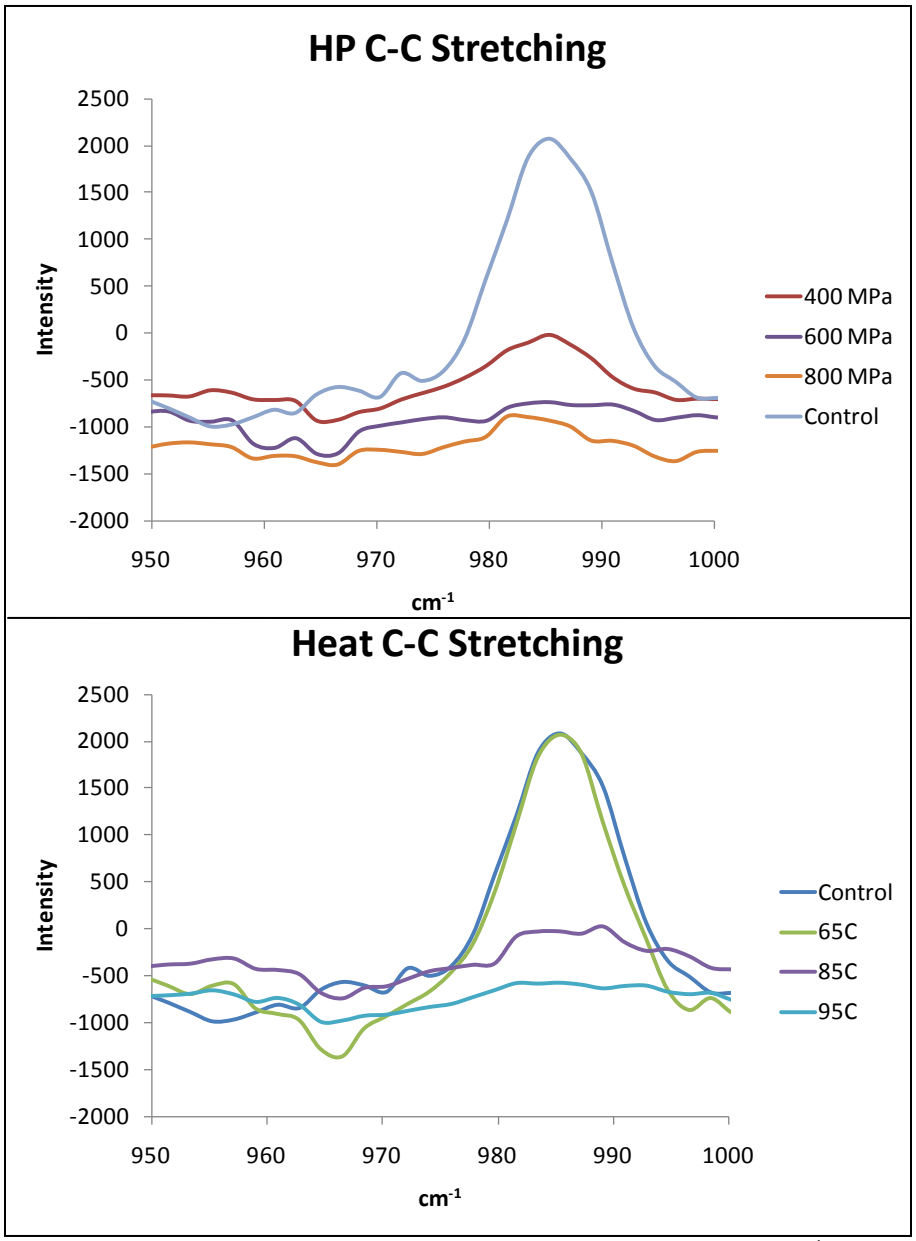

Figure 9: Raman spectra detailing C-C stretching region (950-1000 cm^{-1}) of HP and heat treated egg white. The spectra were auto-baselined with Enwave Optronics software. See methods for measurement and treatment conditions.

The amide I region also revealed changes in secondary structure with a shift to β-sheet (**Figure 10**). An increase of the broad band at 1632-1640 cm^{-1} indicates an increase in intramolecular β-sheet. Both HP and heat treatments also resulted in a shift downfield of the band at 1632-1640 cm^{-1} and shoulder at 1640 cm^{-1}. This shift to higher frequencies is evidence of a loss of α-helix. In addition, the increase of the Raman band at 1624 cm^{-1} corresponds to an increase in exposed β-sheet. The broad α-helical band at 1650-1670 cm^{-1} was not observed in heat or HP-treated egg white. The absence of this band indicates a loss of α-helix. The Raman band at 1670 cm^{-1} arising also indicates an increase in β-sheet.

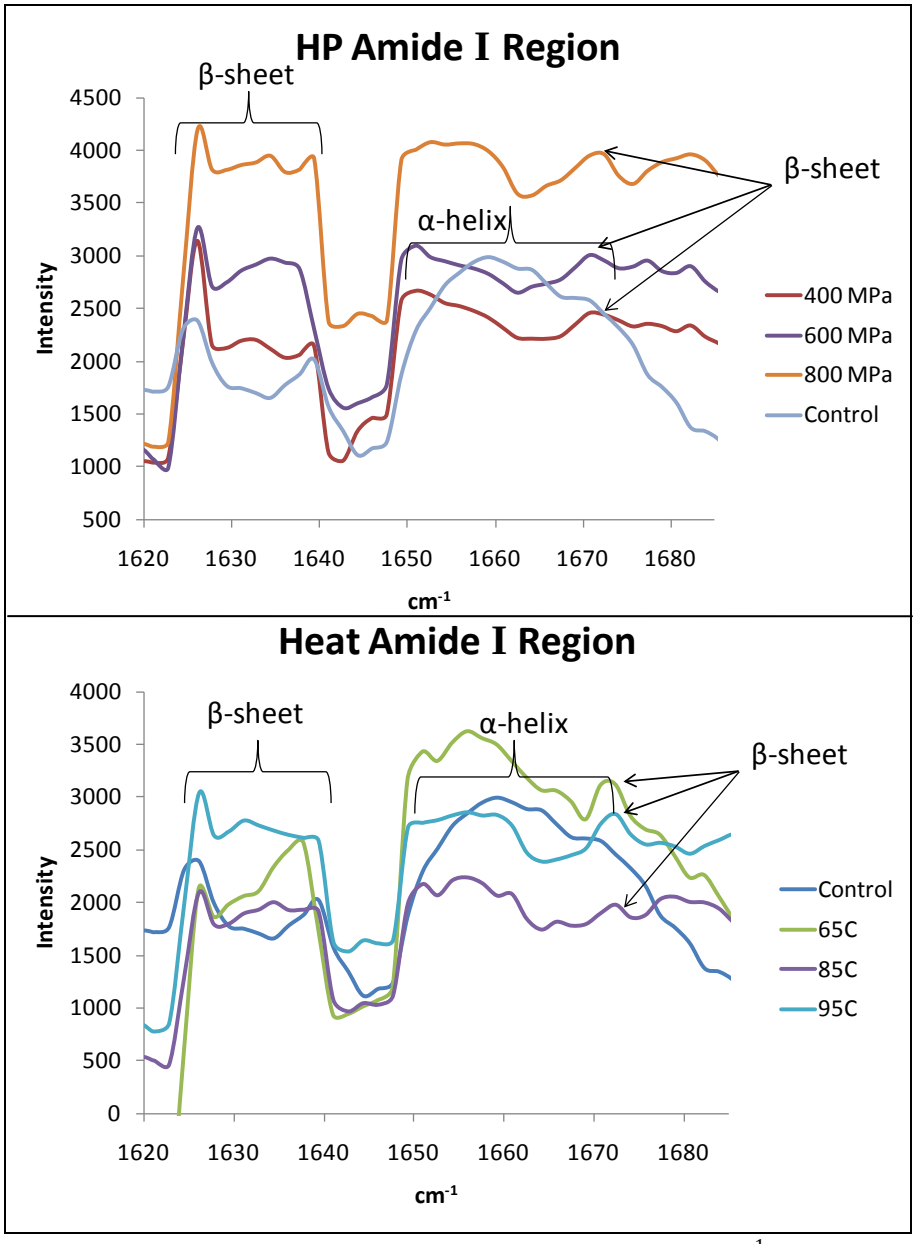

Figure 10: Raman spectra detailing amide I region (1620-1685 cm^{-1}) of HP and heat treated egg white. The spectra were auto-baselined with Enwave Optronics software. See methods for measurement and treatment conditions.

Protein Digestibility

Pepsin digestibility of HP-treated egg white was examined using an in-vitro digestion in simulated gastric fluid. Whole egg white was treated with pressures up to 800 MPa. The control egg white was held at 4°C at ambient pressure. Specific egg white proteins were identified based on migration patterns in the gel gradient. Control egg white was resistant to pepsin digestion, particularly the ovalbumin band (**Figure 11**). Intensity of the ovalbumin band in the control showed little decrease up to 15 min incubation time at a 3:1 pepsin ratio (enzyme: protein). Other investigations (Martos, G. et al 2010) showed resistance of ovalbumin to pepsin digestion at a more physiological enzyme to protein ratio of 1:20. HP- treatment resulted in an increase in pepsin hydrolysis of ovalbumin. Hydrolysis of ovalbumin with digestive enzymes trypsin and chymotrypsin has also been shown to increase with HP-treatment (Iametti, S. et al 1999, Van der Plancken, I. et al 2004). When compared to the control ovalbumin band, pressure treatment of 400 MPa decreased band intensity significantly after 8 min (Lane 8). At 600 MPa, the ovalbumin band is lighter at all time points, with a disappearance of the band observed after 8 min (Lane 8). Increased ovalbumin susceptibility to pepsin digestion was especially evident at 800 MPa with no band observed after 30s (Lane 5). In previous studies, Quiros, A. and others (2007) added pepsin, trypsin, and chymotrypsin before HP-treatment on ovalbumin. This increased the enzymatic hydrolysis of ovalbumin. One key distinction between the approaches of Quiros, A. et al and the work presented in this paper is pepsin digestion of samples was completed post-HP-treatment compared to pre-HP-treatment (Quiros, A. et al 2007).

The ovotransferrin band disappeared after 30s (Lane 5) digestion in all egg white samples indicating ovotransferrin is readily digested by pepsin. The lysozyme band faded in intensity after 2 min (Lane 6) of digestion in the control sample. Mine Y. et al (2004) investigated the enzymatic digestion of egg white lysozyme and also found little resistance to pepsin hydrolysis. Pressures of 600 MPa and greater decreased lysozyme band intensity when compared to the control. The lysozyme band was absent after 30s (Lane 5) at 800 MPa.

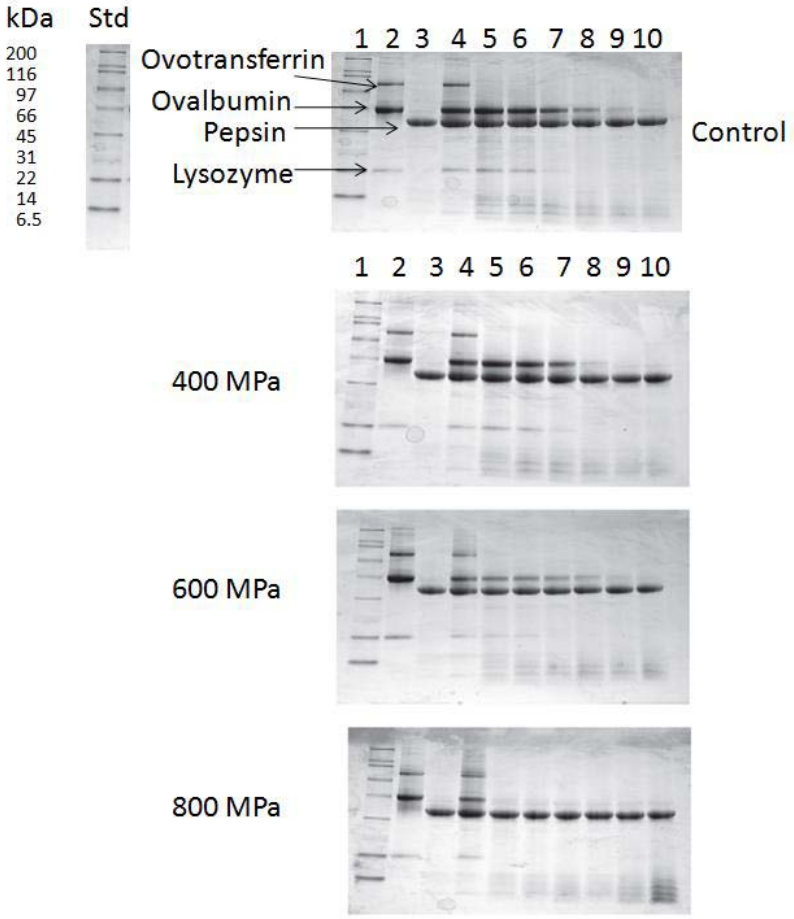

Figure 11: Effects of HP treatment on in-vitro digestion of whole egg white. Samples were pressurized at 400, 600 and 800 MPa for 5 minutes and subsequently digested with pepsin (3:1 enzyme: substrate ratio) in simulated gastric fluid (pH 1.2). SDS-PAGE was performed by loading ~13µg protein for each lane on 10-20% gradient tricine gels. Lanes 1-3 correspond to molecular weight standards, egg white, and pepsin respectively. Lanes 4-10 correspond to digestion incubation times of 0, 30s, 2, 4, 8, 15, and 30 min respectively.

Egg white was also heat treated at temperatures up to 95°C (5 min). Like HP-treatment, thermal treatments increased pepsin digestibility of egg white ovalbumin (**Figure 12**). Degradation of the ovalbumin band was observed after 2-4 min (Lanes 6-7) of digestion at 95°C. However, a faint ovalbumin band remained throughout all incubation times. The ovotransferrin band intensity was decreased after heat treatment before pepsin digestion (Lane 2 and 4). An explanation for the loss in intensity before digestion was attributed to the low denaturation temperature of ovotransferrin (61°C) and subsequent protein degradation. Effects on the lysozyme band were similar to 800 MPa treatments. The lysozyme band on the 85°C gel was also similar to 800 MPa treatments. Treatment at 65°C and 85°C show little difference from control in respect to ovalbumin and ovotransferrin. HP-treatment at 800 MPa resulted in greater digestibility of egg white proteins over thermal treatments (**Figure 12**).

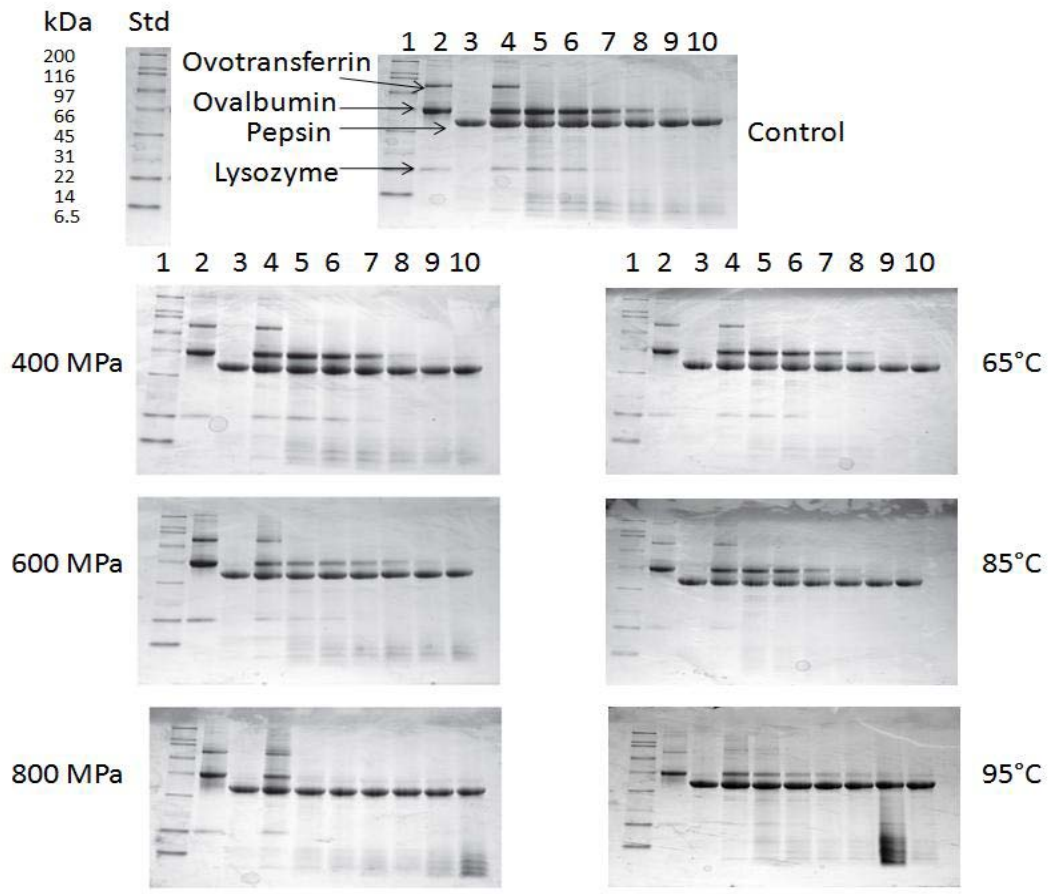

Figure 12: Comparison of HP and heat treated egg white digestion with pepsin and separated by SDS-PAGE as described in **Fig 11**. Samples were pressurized at 400, 600 and 800 MPa for 5 minutes or heated for 5 min at 65, 85, or 95°C. Lanes 1-3 correspond to molecular weight standards, egg white, and pepsin respectively. Lanes 4-10 correspond to digestion incubation times of 0, 30s, 2, 4, 8, 15, and 30 min respectively.

HP-treated egg white pepsin digestibility was also examined at a more physiological enzyme: protein ratio of 1:20. Only control and 800 MPa samples were chosen for this analysis to exhibit the greatest contrast between samples. Two-dimensional electrophoresis was applied for analysis of digestions with an IEF pH range of 3-10 (**Figure 13, 14**). This pH range was chosen as most pI values of major egg white proteins are covered (exception of lysozyme, pI of 10.2). The control and 800 MPa pepsin digestions at time 0 were essentially the same. As incubation time was increased to 15 m, the 800 MPa gel showed increased protein digestion as multiple spots were

evident around pH 4 and 6. These spots mostly corresponded to fragments of ovalbumin (pH 4-5) and ovotransferrin (pH 6) as identified by mass spectrometry analysis (**Table 4**). Specifically, spot 20 (**Fig 13**) was identified as ovotransferrin and its various isoforms. The polymorphisms of ovotransferrin may be attributed to varying degrees of glycosylation or level of bound iron. For instance, the "halo" form of ovotransferrin contains bound iron (Superti, F. et al 2007). The string of spots in line with spot 20 at a lower pH was identified as fragments of ovotransferrin in previous studies (Guerin-Dubiard, C. et al 2006).

Ovalbumin was another protein in which more than one form was identified with mass spectrometry. In many instances (spots 1-3, 5-7, 9, 10), the crystal structure of S-ovalbumin was identified. However, S-ovalbumin properties such as electrophoretic separation are indistinguishable from ovalbumin (Doi, E. et al 1997). A blast comparison between ovalbumin and S-ovalbumin also shows no sequence difference. Its identification cannot be confirmed with electrophoretic analysis but its contribution to spot intensity is possible. S-ovalbumin increases in concentration as egg white ages (Kilara, A. et al 1996) and its presence in the samples is logical as the pH of the egg white used for all experiments (9.1) was above fresh pH (7.6). Other variations of ovalbumin revealed by 2D analysis were ovalbumin related X (spots 3 and 6) and Y proteins (spots 1, 3, 4, 6, 8, 9, 11, 14, 15). The nature of the X and Y related polymorphisms are unexplained other than ovalbumin containing both X and Y genes. However, the sequence homology is identical between X and Y related proteins with X being a fragment of Y (Guerin-Dubiard, C. et al 2006).

Other protein fragments identified were from egg white lysozyme, ovomucoid, protein TENP, Hep21 protein, ovoinhibitors. TENP was first identified in unfertilized egg white by Guerin-Dubiard, C. et al, 2006. TENP is a member of the bactericidal permeability-increasing protein family and is expressed in developing neural tissues (Guerin-Dubiard, C. et al 2006). Like TENP, Hep21 was also recently discovered in hen egg white and has unknown biological activity (Nau, F. et al 2003). Other spots labeled A and B in Control 0 of **Figure 13** correspond to proteins identified in previous proteomic studies of egg white proteins. Spot A can be attributed (assumed to be) to ovalbumin (pI 4.6) and its 3 phosphorylated isoforms, as identified in previous proteomic studies. Spot B can be identified based on previous studies as possible fragments of ovoglycoprotiens and ovomucoid (pI 4.1) (Guerin-Dubiard, C. et al 2006). The control showed increased protein degradation after 30 min of digestion with several spots observed at pH 4-5. At 30 min and 800 MPa intensity of spots present at 15 m was decreased indicating further degradation of protein fragments.

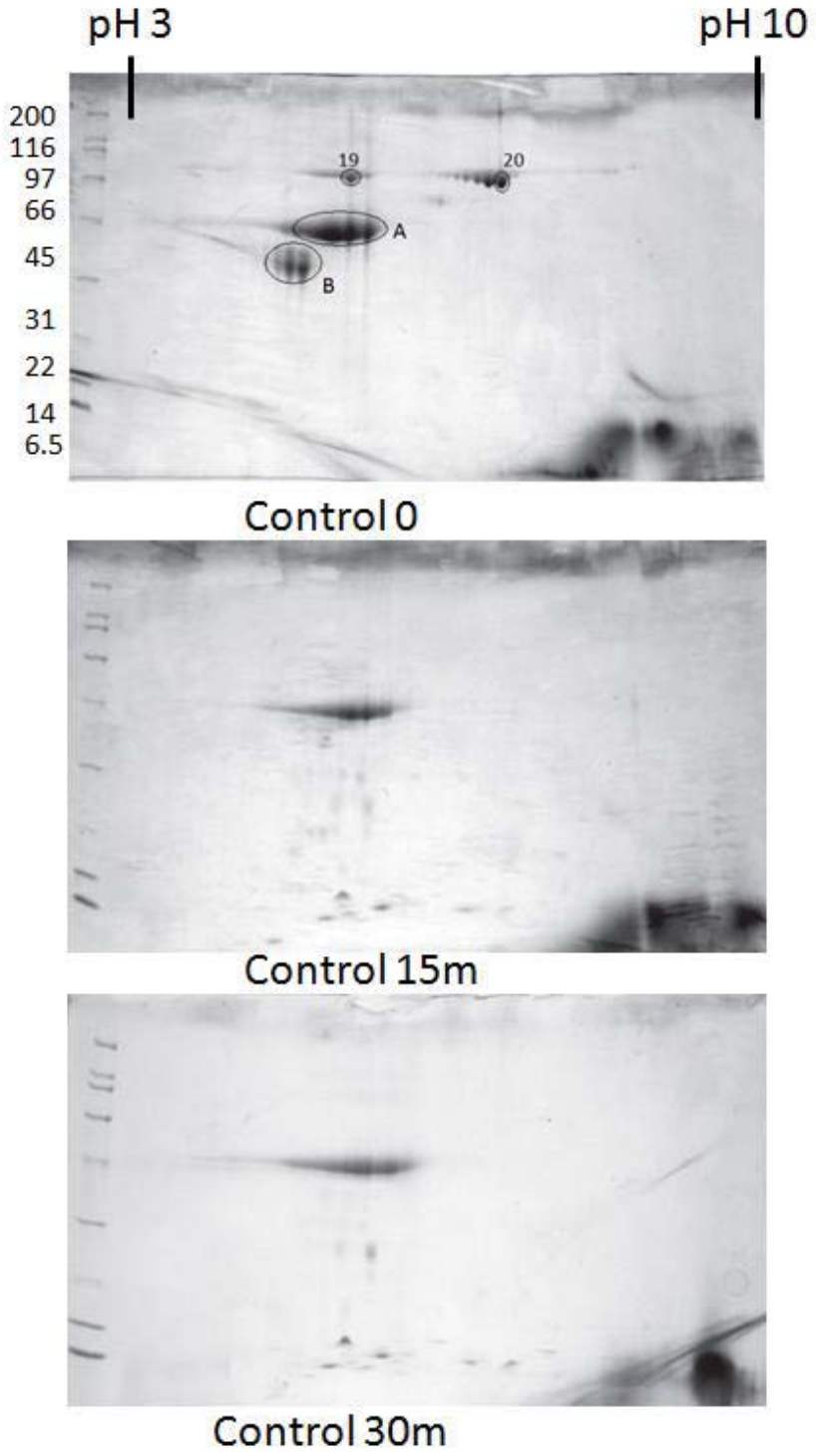

Figure 13: 2-D separation of pepsin digested (1:20 enzyme to protein ratio) control (0.1 MPa) whole egg white in simulated gastric fluid (pH 1.2). Digestions were carried out at 37°C for 0, 15, and 30 min. Protein load was 200 µg and IEF separation was performed on a 3-10 pH gradient for 35k volt hours. Second dimension separation was performed on10-20% gradient Tris-HCl SDS-PAGE gels. Identities of labeled spots were determined by MS sequencing of excised plugs and listed in **Table 4**.

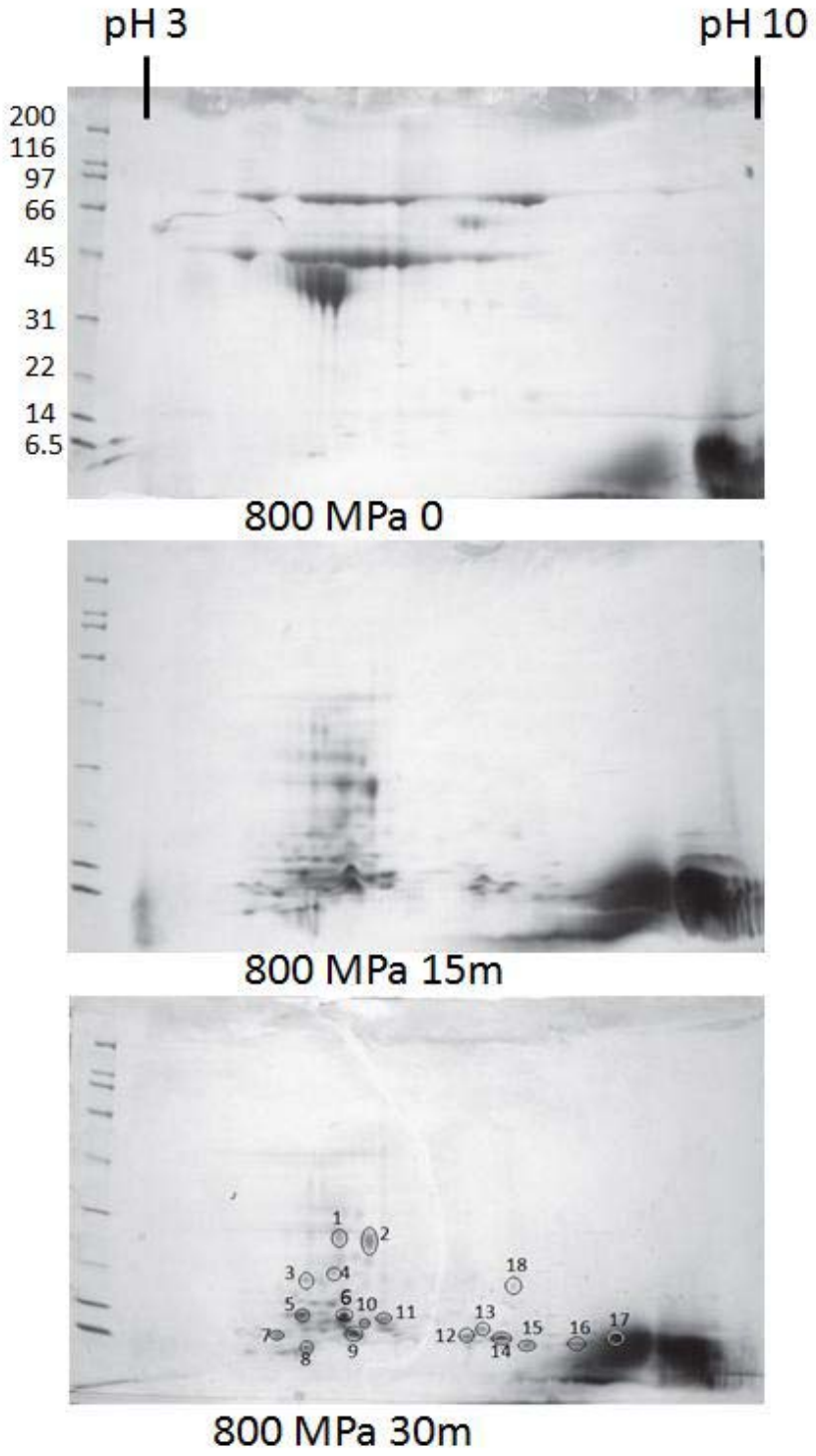

Figure 14: 2-D separation of pepsin digested (1:20 enzyme to protein ratio) 800 MPa treated whole egg white in simulated gastric fluid (pH 1.2). Digestions were carried out at 37°C for 0, 15, and 30 min. Separations were performed on 200 µg aliquots as described in **Fig 13**. Identities of labeled spots were determined by MS sequencing of excised plugs and listed in **Table 4**.

Table 4: Proteins identified via mass spectrometry after 2-D electrophoresis

Spot #	Protein Identification	Gi	Peptides Matches
1	Chain A, Crystal Structure Of S-Ovalbumin	gi|34811330	34
	Ovomucoid	gi|124757	5
	ovalbumin-related protein Y	gi|71897377	1
2	Chain A, Crystal Structure Of S-Ovalbumin	gi|34811330	10
	Ovomucoid	gi|124757	12
	Ovotransferrin	gi|1351295	1
3	Chain A, Crystal Structure Of S-Ovalbumin	gi|34811330	43
	ovalbumin	gi|28566340	39
	Ovalbumin-related protein X	gi|129295	2
	ovalbumin-related protein Y	gi|71897377	1
4	ovalbumin-related protein Y	gi|71897377	3
5	Chain A, Crystal Structure Of S-Ovalbumin	gi|34811330	44
	ovalbumin	gi|28566340	41
	unnamed protein product [Gallus gallus]	gi|63052	12
6	Chain A, Crystal Structure Of S-Ovalbumin	gi|34811330	21
	protein TENP [Gallus gallus]	gi|46048814	4
	Ovotransferrin	gi|1351295	3
	Chain A, Chicken Egg-White Lysozyme Core Mutants	gi|157831883	1
	ovalbumin-related protein Y	gi|71897377	2
	Ovalbumin-related protein X	gi|129295	1
7	Chain A, Crystal Structure Of S-Ovalbumin	gi|34811330	7
	Ovalbumin	gi|129293	7
8	Ovalbumin	gi|129293	7
	ovalbumin N term fragment	gi|223059	5
	ovalbumin-related protein Y	gi|71897377	1
9	Chain A, Crystal Structure Of S-Ovalbumin	gi|34811330	16
	Ovalbumin	gi|129295	1
	ovalbumin-related protein Y	gi|71897377	1
10	Ovalbumin	gi|129293	31
	Chain A, Crystal Structure Of S-Ovalbumin	gi|34811330	31
	Ovotransferrin	gi|1351295	6
	ovotransferrin BB type	gi|71274075	6
	Ovalbumin	gi|129294	10
	protein TENP [Gallus gallus]	gi|46048814	4
	ovoinhibitor precursor	gi|71895337	2
	Chain A, Chicken Egg-White Lysozyme Core Mutants	gi|157831883	1
11	Ovalbumin	gi|129293	29
	Ovotransferrin	gi|1351295	10
	unnamed protein product [Gallus gallus]	gi|63052	17
	protein TENP [Gallus gallus]	gi|46048814	3
	Ovalbumin	gi|129294	2
	ovalbumin N term fragment	gi|223059	3
	ovalbumin-related protein Y	gi|71897377	2
12	Ovotransferrin	gi|1351295	17
	ovotransferrin BB type	gi|71274075	17
	Ovalbumin	gi|129293	12

12	Ovotransferrin	gi|3024757	8
	Lysozyme	gi|126608	2
	Chain A, Chicken Egg-White Lysozyme Core Mutants	gi|157831883	2
	Ovomucoid	gi|124757	4
	protein TENP [Gallus gallus]	gi|46048814	1
13	Ovalbumin	gi|129293	12
	Ovotransferrin	gi|1351295	10
	Ovotransferrin	gi|3024757	6
	Chain A, Chicken Egg-White Lysozyme Core Mutants	gi|157831883	1
	ovoinhibitor precursor	gi|71895337	2
	protein TENP [Gallus gallus]	gi|46048814	1
14	ovotransferrin precursor	gi|45385813	10
	ovotransferrin CC type	gi|71274077	10
	Ovalbumin	gi|129293	7
	protein TENP [Gallus gallus]	gi|46048814	3
	Ovomucoid	gi|124757	2
	Chain A, Chicken Egg-White Lysozyme Core Mutants	gi|157831883	1
	ovalbumin-related protein Y	gi|71897377	1
15	Ovotransferrin	gi|1351295	11
	ovotransferrin BB type	gi|71274075	11
	Ovalbumin	gi|129293	6
	protein TENP [Gallus gallus]	gi|46048814	1
	ovalbumin-related protein Y	gi|71897377	1
16	Ovotransferrin	gi|1351295	7
	Lysozyme	gi|126608	4
	Lysozyme	gi|742827	4
	Chain A, Im Mutant Of Lysozyme	gi|15988033	4
	Chain A, Chicken Egg-White Lysozyme Core Mutants	gi|157831883	2
	unnamed protein product [Gallus gallus]	gi|63052	6
17	Chain A, Aluminum-Bound Ovotransferrin	gi|83754919	24
	ovotransferrin CC type	gi|71274077	22
	Lysozyme	gi|126608	5
	Chain A, Im Mutant Of Lysozyme	gi|15988033	5
	Chain A, Chicken Egg-White Lysozyme Core Mutants	gi|157831883	4
	Lysozyme	gi|742827	3
	unnamed protein product [Gallus gallus]	gi|63052	3
	protein TENP [Gallus gallus]	gi|46048814	1
18	Hep21 protein [Gallus gallus]	gi|45383131	2
	Ovalbumin	gi|129293	4
	Ovotransferrin	gi|1351295	3
	Chain A, Chicken Egg-White Lysozyme Core Mutants	gi|157831883	1
19	Chain A, Aluminum-Bound Ovotransferrin	gi|83754919	79
	Ovotransferrin	gi|1351295	77
	Chain A, Ovotransferrin, N-Terminal Lobe, Holo Form	gi|14719680	36
	Ovalbumin	gi|129293	9
20	Chain A, Aluminum-Bound Ovotransferrin	gi|83754919	99
	ovotransferrin CC type	gi|71274077	94
	Ovotransferrin	gi|1351295	96
	Chain A, Ovotransferrin, N-Terminal Lobe, Holo Form	gi|14719680	46
	Ovotransferrin	gi|3024757	16
	Ovalbumin	gi|129293	2

Bioactive Peptides

In-vitro pepsin digestions (30 min incubation) of control and 800 MPa samples (3:1 ratio) were subject to LC/MS/MS analysis. The analysis was focused on small peptides with a molecular weight <3000 Da. This was accomplished with centrifugation through 3000 Da membrane filters. Samples underwent a clean-up step using Pierce PepClean^{TM} C-18 spin columns and were then dried for LC/MS/MS analysis. The mass spectrometry data was subjected to a Mascot search for possible sequence matches in egg white proteins.

The mass spectrometry analysis of the control and 800 MPa samples had great contrast. No peptide fragment sequences corresponding to egg white proteins were found using standard Mascot search parameters (NCBInr 20100701 metazoa database, significance threshold $p < 0.05$). Identified sequences via Mascot in the control sample were all pepsin derived. The number of proteins matched and unmatched sequences was also substantially less in the control sample. This is another indication of increased pepsin digestibility as a result of pressure treatment. Pepsin digests of 800 MPa treated egg white resulted in peptides from several egg white proteins. The egg white proteins included ovalbumin (gi 28566340), ovotransferrin (gi 83754919), lysozyme mutant (gi 10120553), and protein TENP (gi 46048814). However, none of the Mascot scores were significant to confirm a specific hit based on protein scores. The fragment sequences were still present regardless of the inability to significantly confirm the parent protein.

The egg derived peptide products resulting from 800 MPa pepsin digestion ranged from 9 to 28 amino acids in length. One peptide with possible antihypertensive activity, YAEERYPIL, was identified in the 800 MPa pepsin digested sample. The

origin of YAEERYPIL is ovalbumin (107-115) and has been identified in previous literature with an ACE inhibitor IC_{50} value of 4.7 µM (Miguel, M. et al 2006a; Miguel, M. et al 2007a). YAEERYPIL also exhibits radical scavenging activity of 3.8 µmol of Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) equivalent/µmol of peptide and delays LDL lipid oxidation. Studies by Miguel et al (2006a) of YAEERYPIL show the peptide is further hydrolyzed by pepsin in pancreatic extract to YAEER and YPI. The resulting products do not show as high of activity as YAEERYPIL before peptic digestion with an IC_{50} value of >1000 µg/mL (Miguel, M. et al 2006a). The digestion products of YAEERYPIL were not observed in this analysis. However, this does not mean YAEER or YPI were not present, as these peptides may be beyond the detection limit of the MS analysis performed. This is also true for other potential bioactive peptides as many are < 9 amino acids in length (the smallest length peptide related to egg protein found/identified).

Another sequence with potential ACE-inhibitor bioactivity was found within a fragment identified in the 800 MPa sample (LK**RVPSL**M). The origin of LK**RVPSL**M is ovotransferrin and the sequence, RVPSL (328-332), has been identified in previous literature with an ACE inhibitor IC_{50} value of 20 µM (Liu, J. et al 2010). Further digestion with pepsin using ExPASy Peptide Cutter of LKRVPSLM results in the fragment KRVPSL. Under physiological digestion, trypsin and chymotrypsin would also be present. Theoretically, the bioactive sequence RVPSL could be released with trypsin hydrolysis as it cleaves on the carboxyl side of Lys. However, trypsin would potentially cleave Arg, resulting in the peptide fragment VPSL. A summary of identified digestion products from egg white protein sources are given in **Tables 5-8**.

Ovalbumin contains peptides exhibiting immunomodulating properties which show activity against foreign materials in the body and help in immune function (Wu, J. et al 2010). Two peptides derived from ovalbumin have been reported to show this activity, SVNVHSSL (77-84 residues) and YRGGLEPIN (126-134). Both peptides are currently under investigation for cancer immunotherapy applications and have been shown to increase immune response (Goldberg, et al 2003). One of these peptides, YRGGLEPIN, was embedded in one of the peptide fragments identified in the 800 MPa pepsin digestion sample, L**YRGGLEPIN**F. Again, further hydrolysis by pepsin would theoretically release the bioactive sequence YRGGLEPIN as predicted by ExPASy Peptide Cutter. The results presented suggest HPP has the potential to increase release of bioactive peptides in egg white proteins and be beneficial to egg white nutritional value.

Table 5: Pepsin digestion products identified from 800 MPa treated egg white corresponding to ovalbumin. Amino acids before and after identified sequence are given.

Mass Exp	**Mass Theo**	**Sequence**	**Ovalbumin residue**
894.53	894.43	A.MPFRVTE.Q + Oxidation (M)	197-203
1035.49	1035.52	Q.ITKPNDVYS.F	91-99
1076.51	1076.5	E.QESKPVQMM.Y	204-212
1099.53	1099.45	A.FKDEDTQAM.P + Oxidation (M)	189-197
1152.63	1152.58	L.YAEERYPIL.P	107-115
1160.58	1160.65	M.ASEKMKILEL.P	224-233
1176.61	1176.57	F.DKLPGFGDSIE.A	61-71
1182.46	1182.59	Q.ITKPNDVYSF.S	91-100
1193.57	1193.56	F.EKLTEWTSSN.V	263-272
1209.69	1209.64	M.VYLGAKDSTRT.Q	42-52
1229.68	1229.64	W.VESQTNGIIRN.V	150-160
1277.6	1277.68	E.LYRGGLEPINF.Q	125-135
1277.65	1277.63	L.NQITKPNDVYS.F	89-99
1277.74	1277.68	E.LYRGGLEPINF.Q	125-135
1282.7	1282.59	I.LELPFASGTMSM.L	231-242
1305.68	1305.68	L.SGISSAESLKISQ.A	314-326
1305.78	1305.7	M.KILELPFASGTM.S	229-240
1339.81	1339.75	G.IIRNVLQPSSVD.S	157-168
1415.74	1415.72	S.WVESQTNGIIRN.V	149-160
1424.7	1424.69	L.NQITKPNDVYSF.S	89-100
1430.82	1430.76	F.KELKVHHANENI.F	19-28
1450.86	1450.78	M.VYLGAKDSTRTQI.N	42-54
1523.89	1523.77	M.KILELPFASGTMSM.L	229-242
1554.81	1554.84	G.IIRNVLQPSSVDSQ.T	157-170

1564.83	1564.82	M.VYLGAKDSTRTQIN.K	42-55
1616.89	1616.83	L.KISQAVHAAHAEINE.A	323-337
1692.98	1692.92	M.VYLGAKDSTRTQINK.V	42-56
1726.92	1726.92	G.IIRNVLQPSSVDSQTA.X	157-172
1812.93	1812.84	A.FKDEDTQAMPFRVTE.Q	189-203
1852.09	1851.95	M.ASEKMKILELPFASGTM.S	224-240
1857.95	1857.96	G.IIRNVLQPSSVDSQTAM.V	157-173
1874.06	1873.96	G.IIRNVLQPSSVDSQTAM.V + Oxidation (M)	157-173
1941	1940.89	A.FKDEDTQAMPFRVTEQ.E	189-204
2332.21	2332.17	W.VESQYNGIIRNVLQPSSVDSQ.T	150-170
2679.62	2679.5	G.IIRNVLQPSSVDSQTAPVLVNAIVF.K	157-181

Table 6: Pepsin digestion products identified from 800 MPa treated egg white corresponding to ovotransferrin. Amino acids before and after identified sequence are given.

Mass Exp	**Mass Theo**	**Sequence**	**Ovotransferrin residue**
894.45	894.44	F.LSKAQSDF.G	267-274
942.6	942.57	M.LKRVPSLM.D	307-314
1145.67	1145.58	T.YKEFLGDKF.Y	653-661
1165.62	1165.56	F.LSKAQSDFGVD.T	267-277
1207.61	1207.5	E.NAPDQKDEYE.L	216-225
1340.83	1340.72	L.GRSAGWNIPIGTL.I	120-132
1403.82	1403.79	F.HLFGPPGKKDPVL.K	283-295
1440.88	1440.83	F.EAGLAPYKLKPIAA.E	66-79
1456.82	1456.75	S.MRKDQLTPSPRE.N	331-342
1501.71	1501.62	A.EVYEHTEGSTTSY.Y	80-92
1522.79	1522.89	A.VVVRPEKANKIRD.L	594-606
1569.97	1569.88	F.EAGLAPYKLKPIAAE.V	66-80
1816.14	1816.01	Q.VFEAGLAPYKLKPIAAE.V	64-80
1823.97	1823.86	F.VKHTTVNENAPDQKDE.Y	208-223
1987.06	1986.93	F.VKHTTVNENAPDQKDEY.E	208-224
2116.11	2115.97	F.VKHTTVNENAPDQKDEYE.L	208-225
2229.18	2229.05	F.VKHTTVNENAPDQKDEYEL.L	208-226

Table 7: Pepsin digestion products identified from 800 MPa treated egg white corresponding to lysozyme mutant. Amino acids before and after identified sequence are given.

Mass Exp	**Mass Theo**	**Sequence**	**Lysozyme mutant residue**
1656.83	1656.7	F.NTQATNRNTDGSTDY.G	39-53
1939.97	1939.89	F.NTQATNRNTDGSTDYGIL.Q	39-56
3313.66	3313.48	F.NTQATNRNTDGSTDYGILQINSRWWCNDA.R	39-67

Table 8: Pepsin digestion products identified from 800 MPa treated egg white corresponding to protein TENP. Amino acids before and after identified sequence are given.

Mass Exp	**Mass Theo**	**Sequence**	**protein TENP**
1099.63	1099.55	L.YHEDLPITL.S	288-296
1353.84	1353.74	A.VVPVPVSPVPFSM.P	219-231
1780.09	1779.96	F.QVAGAVVPVPVSPVPFSM.P	214-231
2501.47	2501.35	L.DKVVDVDKLCLDVSKLLLFPNE.Q	152-173

The fragments isolated in this work were overlaid on the sequences of common egg white allergens ovalbumin, ovotransferrin, and lysozyme (**Figure 15**). Select ovalbumin allergenic epitope sequences are highlighted in yellow. The highlighted ovalbumin sequences correspond to epitope regions with distinct IgE recognition in human patients with secondary structure that has been determined (Mine, Y. et al 2003). The change in secondary structure due to HP-treatment as determined by Raman analysis may have an effect on the allergenicity (IgE binding) of these epitopes. Other reported allergenic epitopes in human patients correspond to ovalbumin sequence 2-11, 12-20, 34-78, 42-172, 302-286, and 348-386 (Mine, Y. et al 2008). Many of these epitopes are overlapping and when combined, have extensive sequence coverage. **Figure 15** illustrates the fragmentation of epitope sequences as a result of pressure treatment and subsequent pepsin digestion. As reported previously, HP-treatment has the potential to reduce allergenicity (IgE binding) by increasing proteolysis (Lopez-Exposito, I. et al 2008). The rationale behind this conclusion is the fact that most food egg white protein epitopes are sequential and thus by increasing proteolysis it may reduce allergenic response (IgE binding) (Mine, Y. et al 2008). No specific epitopes have been characterized in human studies relating to egg allergy for ovotransferrin or lysozyme.

Ovalbumin

```
  1 MGSIGAASME FCFDVFKELK VHHANENIFY CPIAIMSALA MVYLGAKDST
 51 RTQINKVVRF DKLPGFGDSI EAQCGTSVNV HSSLRDILNQ ITKPNDVYSF
101 SLASRLYAEE RYPILPEYLQ CVKELYRGGL EPINFQTAAD QARELINSWV
151 ESQXNGIIRN VLQPSSVDSQ TAXVLVNAIV FKGLWEKAFK DEDTQAMPFR
201 VTEQESKPVQ MMYQIGLFRV ASMASEKMKI LELPFASGTM SMLVLLPDEV
251 SGLEQLESII NFEKLTEWTS SNVMEERKIK VYFPRMKMEE KYNLTSVLMA
301 MGITDVFSSS ANLSGISSAE SLKISQAVHA AHAEINEAGR EVVGSAEAGV
351 DAASVSEEFR ADHPFLFCIK HIATNAVLFF GRCVSP
```

Ovotransferrin

```
  1 APPKSVIRWC TISSPEEKKC NNLRDLTQQE RISLTCVQKA TYLDCIKAIA
 51 NNEADAISLD GGQVFEAGLA PYKLKPIAAE VYEHTEGSTT SYYAVAVVKK
101 GTEFTVNDLQ GKTSCHTGLG RSAGWNIPIG TLIHRGAIEW EGIESGSVEQ
151 AVAKFFSASC VPGATIEQKL CRQCKGDPKT KCARNAPYSG YSGAFHCLKD
201 GKGDVAFVKH TTVNENAPDQ KDEYELLCLD GSRQPVDNYK TCNWARVAAH
251 AVVARDDNKV EDIWSFLSKA QSDFGVDTKS DFHLFGPPGK KDPVLKDLLF
301 KDSAIMLKRV PSLMDSQLYL GFEYYSAIQS MRKDQLTPSP RENRIQWCAV
351 GKDEKSKCDR WSVVSNGDVE CTVVDETKDC IIKIMKGEAD AVALDGGLVY
401 TAGVCGLVPV MAERYDDESQ CSKTDERPAS YFAVAVARKD SNVNWNNLKG
451 KKSCHTAVGR TAGWVIPMGL IHNRTGTCNF DEYFSEGCAP GSPPNSRLCQ
501 LCQGSGGIPP EKCVASSHEK YFGYTGALRC LVEKGDVAFI QHSTVEENTG
551 GKNKADWAKN LQMDDFELLC TDGRRANVMD YRECNLAEVP THAVVVRPEK
601 ANKIRDLLER QEKRFGVNGS EKSKFMMFES QNKDLLFKDL TKCLFKVREG
651 TTYKEFLGDK FYTVISSLKT CNPSDILQMC SFLEGK
```

Lysozyme

```
  1 KVFGRCELAA AMKRHGLDNY RGYSLGNWVC AAKFESNFNT QATNRNTDGS
 51 TDYGILQINS RWWCNDARTP GSRNLCNIPC SALLSSDITA SVNCAKKIVS
101 DGNGMNAWVA WRNRCKGTDV QAWIRGCRL
```

Protein TENP

```
  1 MGALLALLDP VQPTRAPDCG GILTPLGLSY LAEVSKPHAE VVLRQDLMPK
 51 EPQTCSLAPW SPAGTELPAV KVADLWLSVI PEAGLRLGIE VELRIAPLHT
101 VPMPVRISIR ADLHVDMGPD GNLQLLTSAC RPTVQAQSTR EAESKSSRSI
151 LDKVVDVDKL CLDVSKLLLF PNEQLMSLTA LFPVTPNCQL QYLALAAPVF
201 SKQGIALSLQ TTFQVAGAVV PVPVSPVPFS MPELASTSTS HLILALSEHF
251 YTSLYFTLER AGAFNMTIPS MLTTATLAQK ITQVGSLYHE DLPITLSAAL
301 RSSPRVVLEE GRAALKLFLT VHIGAGSPDF QSFLSVSADV TRAGLQLSVS
351 DTRMMISTAV IEDAELSLAA SNVGLVRAAL LEELFLAPVC QQVPAWMDDV
401 LREGVHLPHM SHFTYTDVNV VVHKDYVLVP CKLKLRSTMA
```

Figure 15: Sequence coverage of identified peptides in 800 MPa pepsin digested sample. Matched sequences are shown in red. Highlighted yellow areas indicate identified ovalbumin allergenic (IgE) epitopes with distinct IgE recognition reported in human egg-allergenic persons (Mine, Y. et al 2003).

Egg White Functionality

Gel Texture and Color

Texture properties of HP-treated (600 and 800 MPa) and heat-treated (95°C) egg white gels were investigated as described in the methods. Samples adjusted to pH 6 with tartaric acid prior to HP or thermal treatments were also analyzed. Textural profile analysis was applied as described by Bourne (1982) to determine gel hardness, gumminess, cohesiveness, and resilience. The texture profile consists of a two bite cycle which produces an output of two peaks (**Figure 16**).

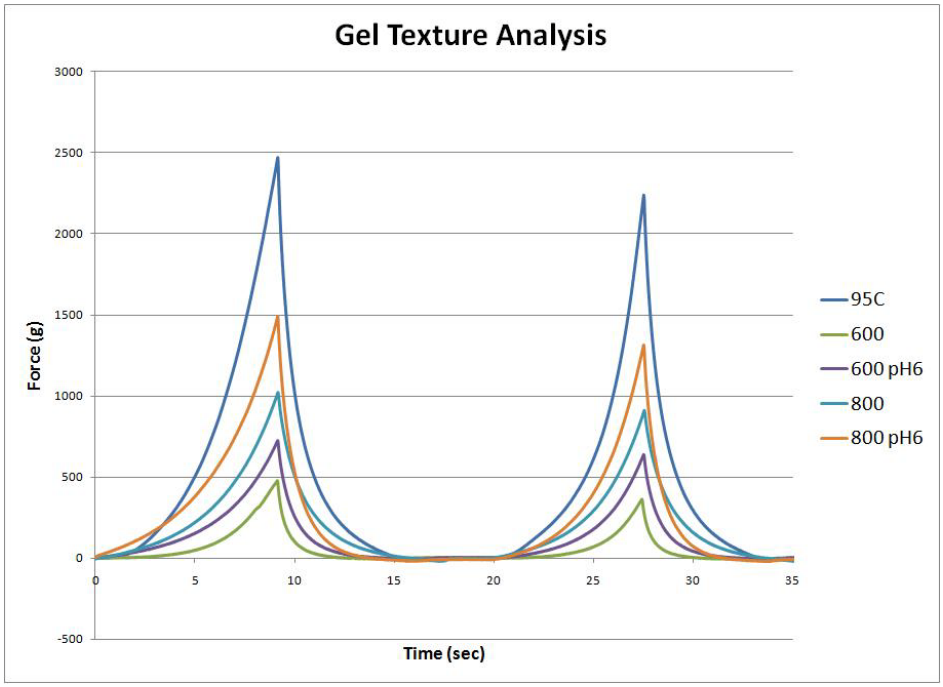

Figure 16: Texture profile of representative runs for samples treated at 95°C, 600 MPa and 800 MPa at natural pH (9.11). Also included are profiles for whole egg white adjusted to pH 6 with tartaric acid and treated at 600 and 800 MPa.

Gels formed with heat at 95°C had an average hardness value of 2329 g, over twice the value (1114.89 g) of HP-induce gels at 800 MPa (**Figure 17**). The softest gel was observed at 600 MPa. Reduction of pH decreased the hardness of heat induced gels while it increased the hardness of HP gels. Gel gumminess followed a similar pattern to gel hardness with heat induced gels being gummier (**Figure 18**).

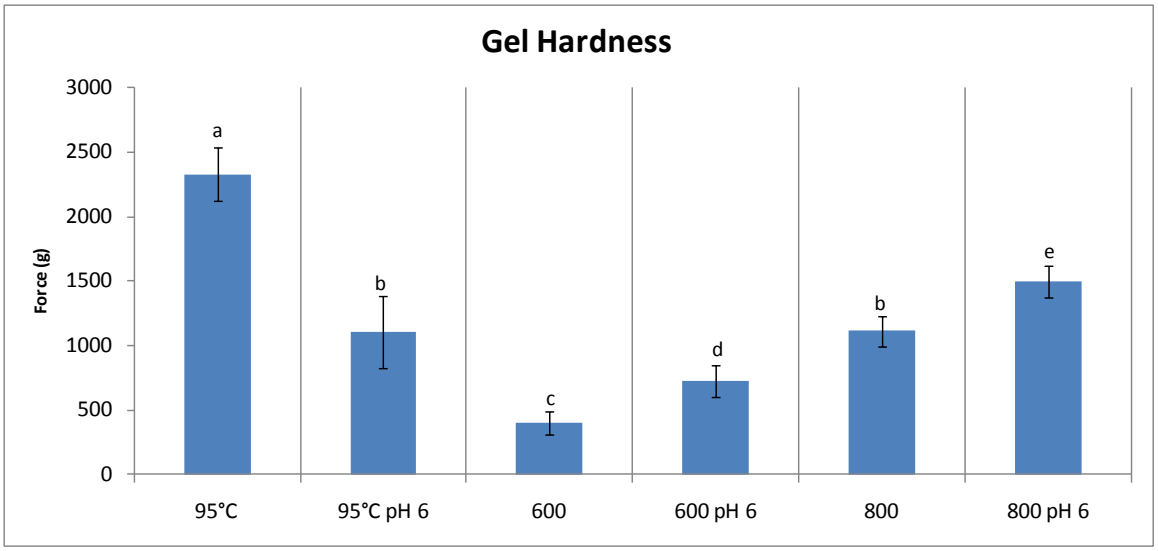

Figure 17: Effect of heat and HP-treatment on egg white gel hardness at natural pH (9.11) and pH adjusted with tartaric acid (pH 6.0). Same letters above each bar for each sample denote means are not significantly different (α=0.05) using Duncan's test and Dunnet least square means. Error bars are ± 1 standard deviation

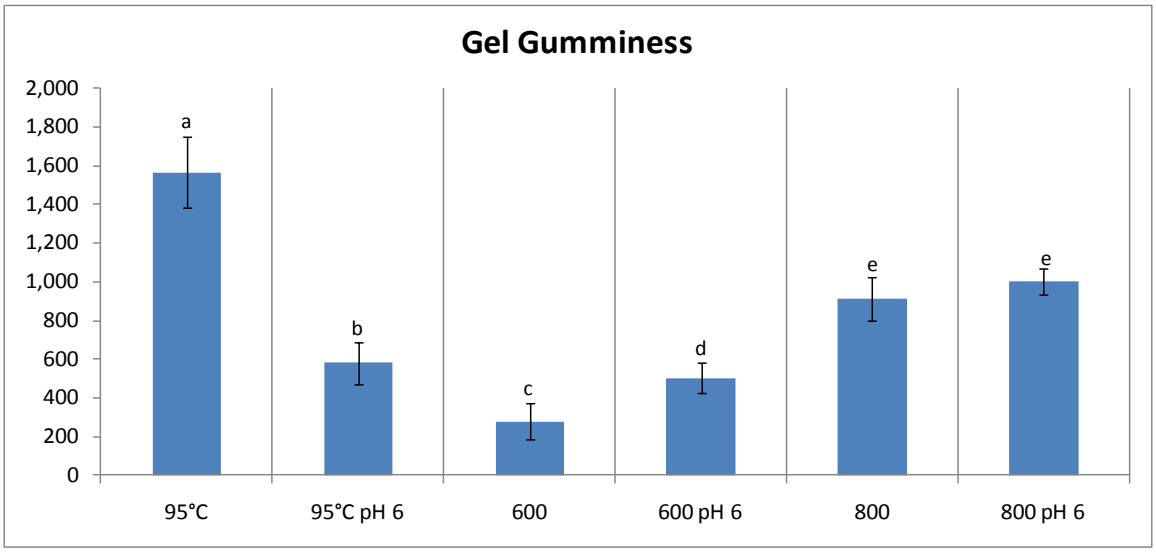

Figure 18: Effect of heat and HP-treatment on egg white gel gumminess at natural pH (9.11) and pH adjusted with tartaric acid (pH 6.0). Same letters above each bar for each sample denote means are not significantly different (α=0.05) using Duncan's test and Dunnet least square means. Error bars are ± 1 standard deviation.

Gels formed with HP at 800MPa were more resilient (instant springiness) than heat induced gels (**Figure 19**). Resilience decreased as pH was decreased at 800 MPa. However, lowered pH increased resilience at 600 MPa. This may be explained by the softness and almost jelly like state (semi-solid) of the 600 MPa gel which could prevent

instant springiness/bounce back. Extended error bars for 600 MPa may also be explained by its semi-solid state. Resilience of heat induced gels was decreased at pH 6.0. Gel cohesiveness showed little difference between treatments with HP-induced gels having slightly higher values (**Figure 20**).

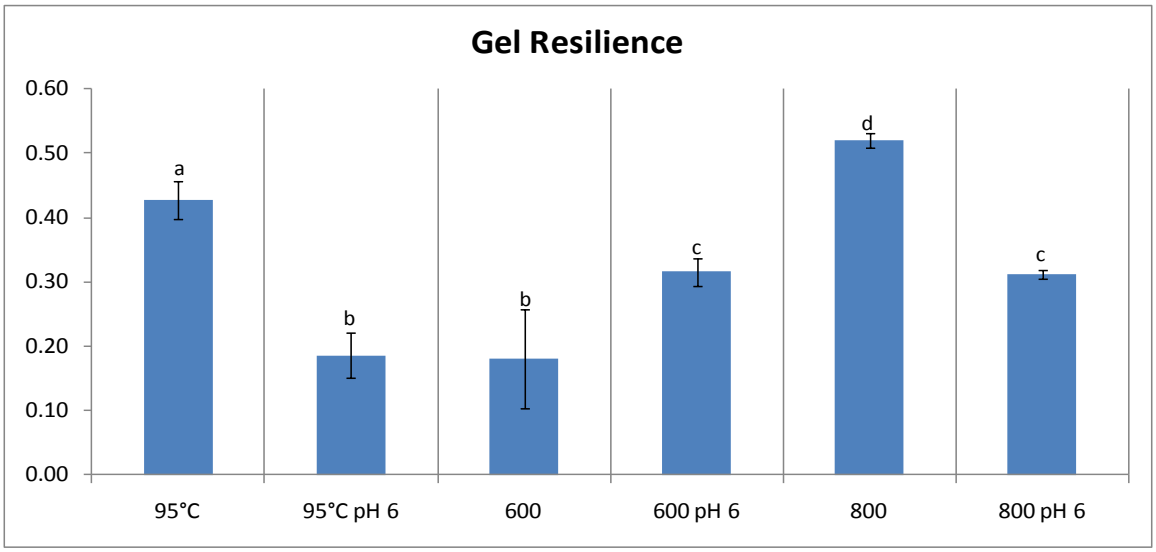

Figure 19: Effect of heat and HP-treatment on egg white gel resilience at natural pH (9.11) and pH adjusted with tartaric acid (pH 6.0). Same letters above each bar for each sample denote means are not significantly different (α=0.05) using Duncan's test and Dunnet least square means. Error bars are ± 1 standard deviation.

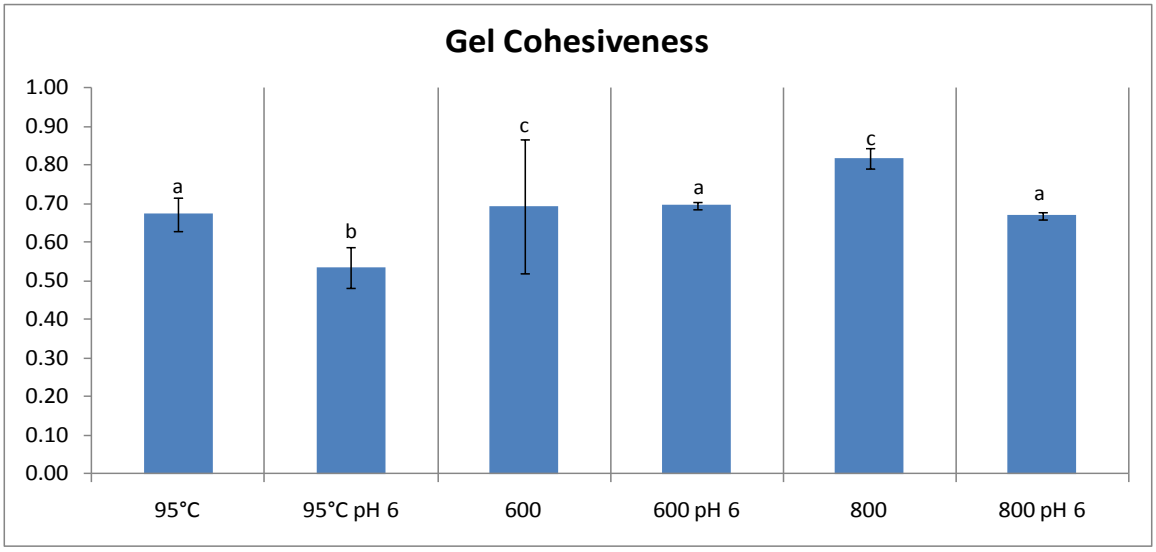

Figure 20: Effect of heat and HP-treatment on egg white gel cohesiveness at natural pH (9.11) and pH adjusted with tartaric acid (pH 6.0). Same letters above each bar for each sample denote means are not significantly different (α=0.05) using Duncan's test and Dunnet least square means. Error bars are ± 1 standard deviation.

As in previous studies (Hayashi, R. et al 1989; Ngarize, S. et al 2005), HP-induced gels were softer and more elastic than thermally induced gels. The textural properties of heat-induced gels tended to have greater measurement variation, particularly the hardness and gumminess properties. These variations may be explained by uneven heat transfer during treatment or insufficient cooking time (softer near the middle of sample). In this respect, HP-induced gels were more uniform. Lowering the pH changed the appearance of egg white gels as they were more granular (**Figure 21**)

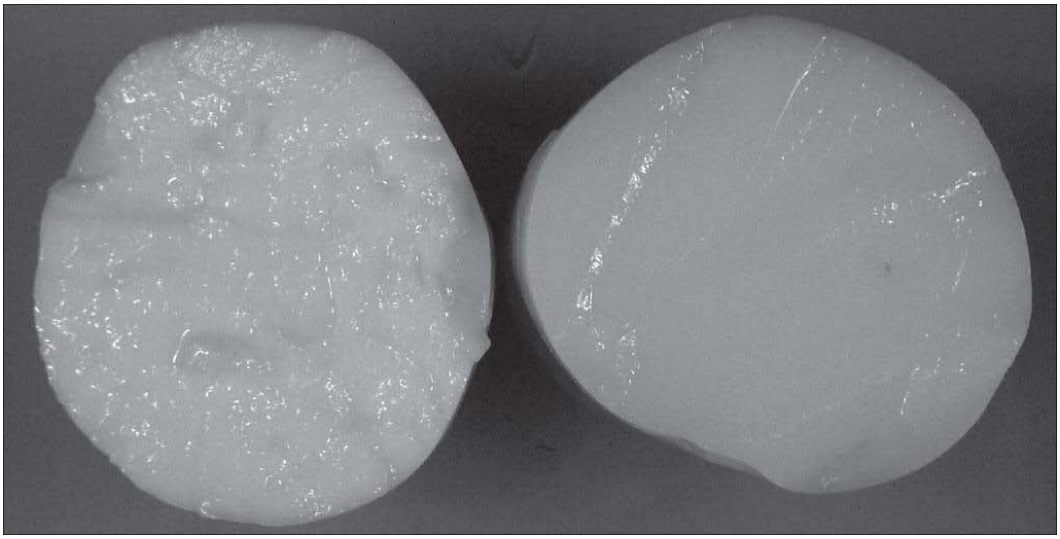

Figure 21: Cross section of pH 9.11 and 6.0 800 MPa treated gels.

Gel syneresis was observed in all samples. Heat-induced gels noticeably decreased in diameter/size once cooled after treatment. This was expected as others have reported shrinkage of heat-induced gels due to lowered water holding capacity (Croguennec, T. et al 2002, Nakamura, R. et al 2000). Liquid discharged from the gel was subject to SDS-PAGE analysis (**Figure 22**). Lysozyme bands were absent from the discharge liquid. This indicates it was incorporated in the gel matrix. The ovotransferrin band was present, indicating it may be less important to gel structure. The ovalbumin

band decreased in intensity as pressure was increased. Thus, as pressure was increased ovalbumin was further incorporated in the gel matrix.

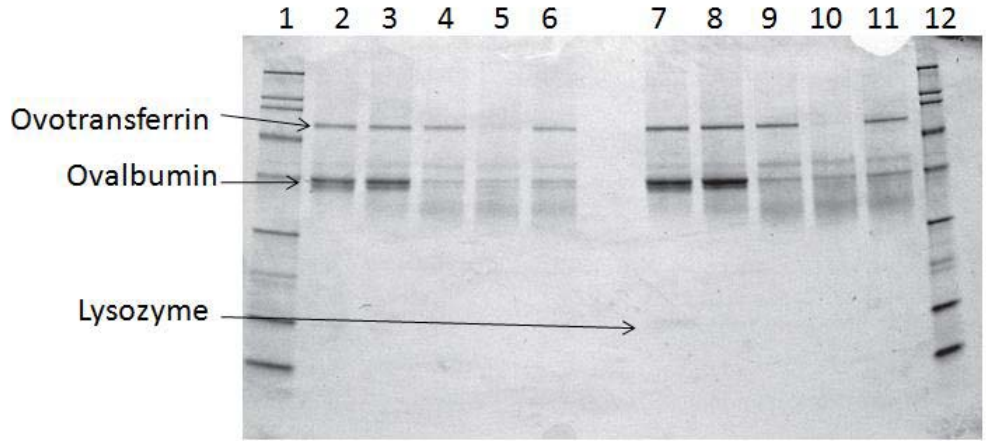

Figure 22: SDS-PAGE analysis of gel syneresis liquid using 10-20% gradient Tricine gels. Lanes 1-6 (10 µg load) correspond to molecular weight standards, control, 600 MPa, 600 MPa pH 6, 800 MPa, 800 MPa pH 6 respectively. Lanes 7-12 have a protein load of 20 ug.

The color of egg gels was analyzed and compared using the Hunter color scale (**Figure 23**). Heat treated gels (95°C, 10 min) had a significantly higher L-value than HP gels (α=0.05). Increasing levels of pressure did not significantly affect L-values. Lowering the pH to 6.0 with tartaric acid improved overall gel appearance, resulting in brighter/more white gels with both heat and HP gels (**Figure 24**).

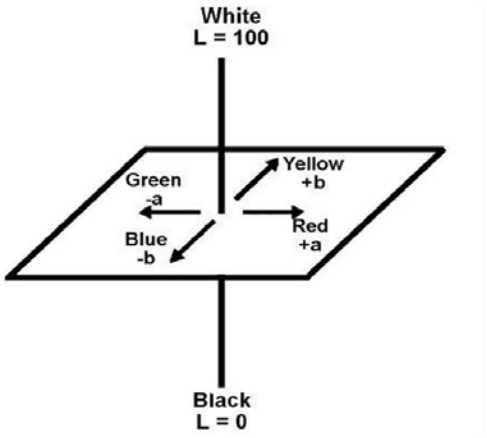

Figure 23: Hunter L, a, b color scale

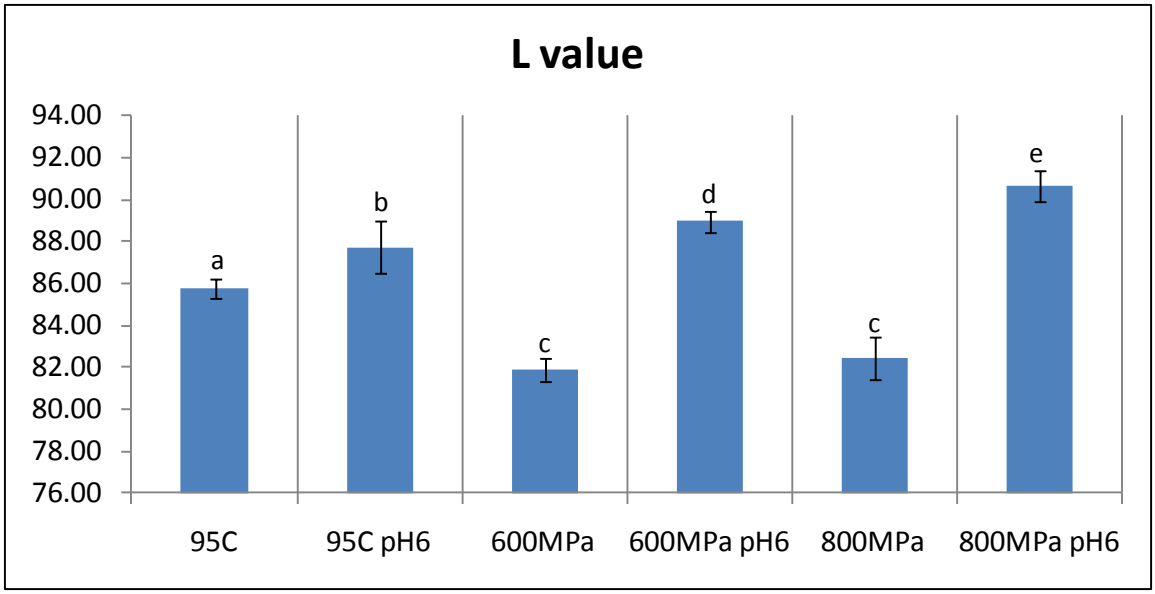

Figure 24: Effect of heat and HP-treatment on egg white color L values at natural pH (9.11) and pH adjusted with tartaric acid (pH 6.0). Same letters above each bar for each sample denote means are not significantly different (α=0.05) using Duncan's test and Dunnet least square means. Error bars are ± 1 standard deviation.

All egg white gels produced negative a-values which indicate a greener color according to the Hunter scale. Heat-induced gels had the lowest a-value at -4.55. This was closely followed by the 800 MPa gel (-4.10). Lowering the pH resulted in significantly increased a-values (**Figure 25**). With respect to b-values (**Figure 26)**, lowering the pH significantly increased the value indicating a more yellow color. Heat-induced gels also had a higher b-value than HP-gels. Although 'a' and b-values indicate the presence of other colors, all gels were visually white.

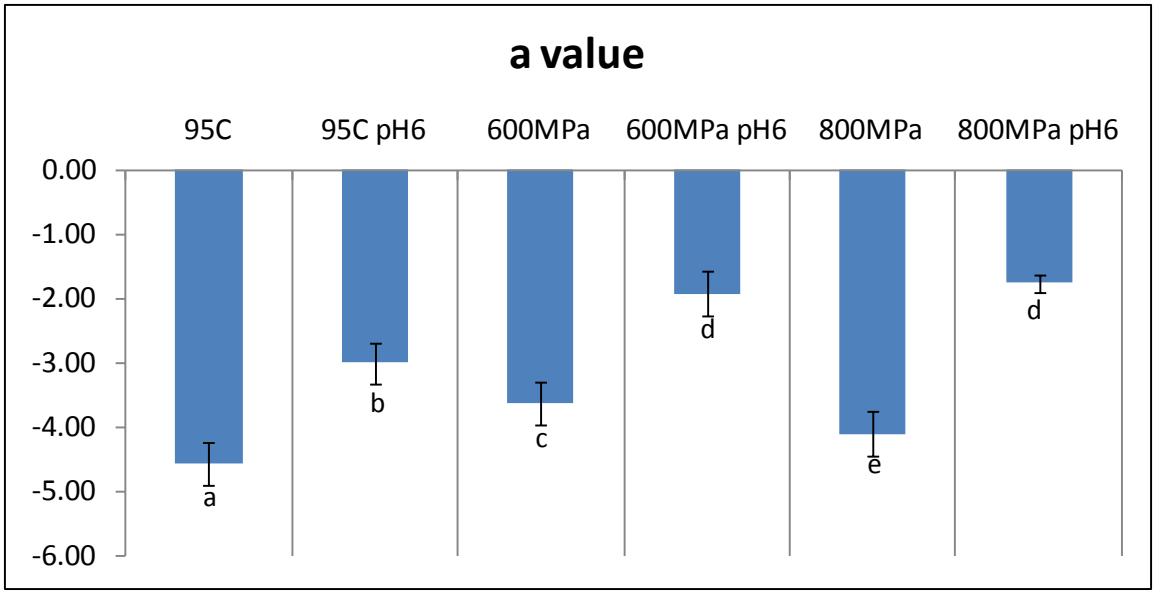

Figure 25: Effect of heat and HP-treatment on egg white color a values at natural pH (9.11) and pH adjusted with tartaric acid (pH 6.0). Same letters above each bar for each sample denote means are not significantly different (α=0.05) using Duncan's test and Dunnet least square means. Error bars are ± 1 standard deviation.

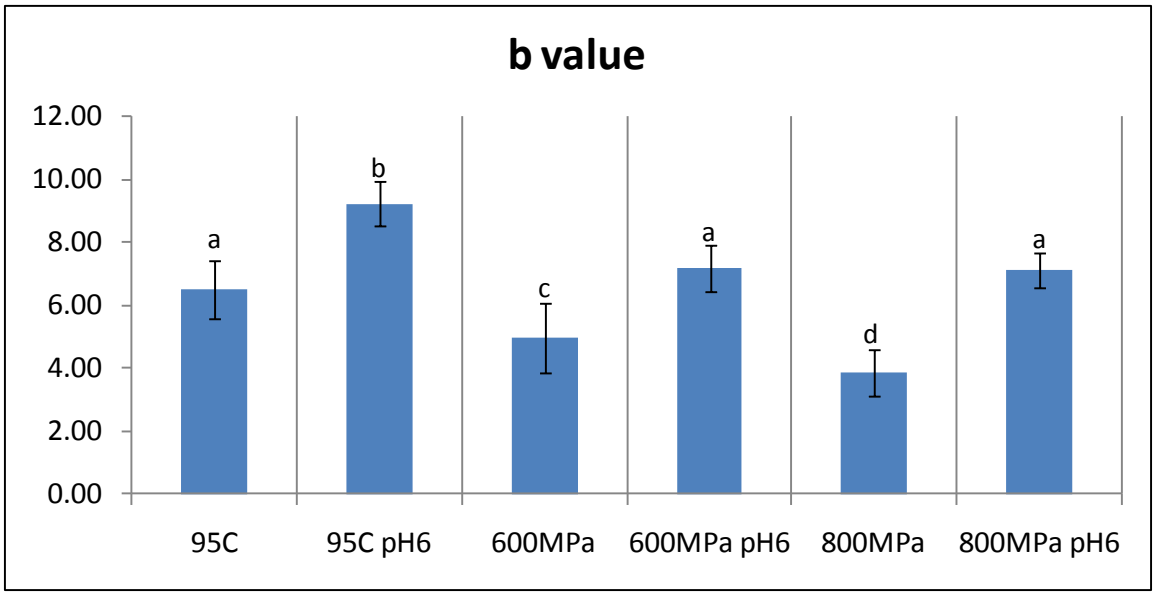

Figure 26: Effect of heat and HP-treatment on egg white color a values at natural pH (9.11) and pH adjusted with tartaric acid (pH 6.0). Same letters above each bar for each sample denote means are not significantly different (α=0.05) using Duncan's test and Dunnet least square means. Error bars are ± 1 standard deviation.

Foaming Properties

Foaming properties of egg white solutions (10% v/v) were analyzed with varying levels of pressure and pH as described in the methods. Whole egg white was not used for foaming analysis due to pressure induced gelation at 600 and 800 MPa. Thus, all egg white was diluted to 10% with RO water. Only the natural pH (9.11) of the egg white was used in pressure treatments as egg white solutions of pH 6.0 and pH 4.5 resulted in protein precipitation after HPP.

Pressure treatment of 10% egg white solutions at pH 9.11 resulted in a homogenous solution with improved foaming capacity over the control (see **Figure 27**). Increasing pressure resulted in an increase in foam volume. Foam overrun was significantly ($\alpha = 0.05$) increased at 800 MPa at all time points. At 5 min and 30 min there was no significant difference in foam overrun between 600 and 800 MPa. Foam overrun was slightly increased at 600 MPa, but this increase was not statistically significant. Van der Plancken et al (2007a) also found HP treatment resulting in increased foam capacity. Increased foam overrun with HPP can be attributed to partial unfolding of egg white protein associated with pressure which contributes to absorption into air-water interface.

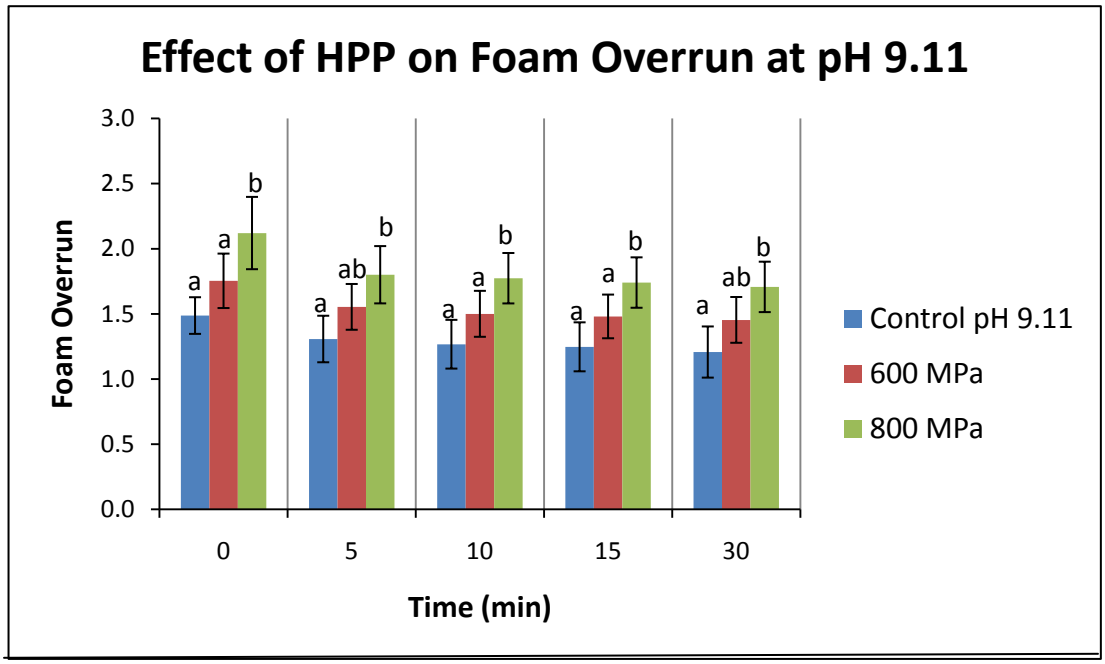

Figure 27: Effect of HPP (5 min) on 10% egg white solution foam overrun at pH 9.11 and at 0.1 MPa (control), 600 MPa, and 800 MPa. Time intervals represent measurement of foam overrun post-foaming. Same letters above each bar for each time point denote means are not significantly different (α=0.05) using Duncan's test and Dunnet least square means. Error bars are ± 1 standard deviation.

The foaming properties of egg white solutions were also highly dependent on pH. The greatest foam overrun was achieved at pH 4.5 while foaming ability was significantly decreased at pH 6 (**Figure 28)**. The increased foam overrun at pH 4.5 could be attributed to major egg white proteins important to foaming properties, ovalbumin and ovomucin, which have respective pI of 4.5 and 4.1. At pH 4.5, the two proteins are uncharged resulting in a reduction of electrostatic repulsion between protein molecules. This allows for easier absorption into the air-water interface when foamed due to easier protein unfolding. The decrease in foam overrun at pH 6 may be due to increased electrostatic protein-protein interactions, which would decrease absorption into the air-water interface.

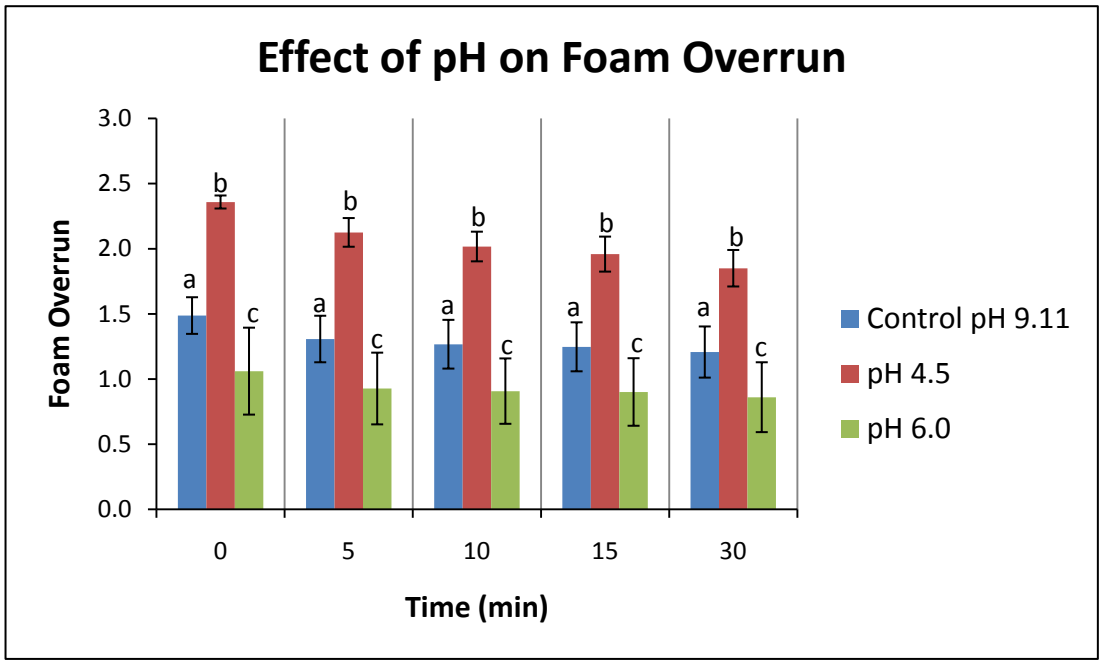

Figure 28: Effect of pH on 10% egg white solution foam overrun at pH 9.11 (control), pH 6.0, and pH 4.5 at room temperature (20°C). Time intervals represent measurement of foam overrun post-foaming. Same letters above each bar for each time denote means are not significantly different (α=0.05) using Duncan's test and Dunnet least square means. Error bars are ± 1 standard deviation.

Foam stability was also used to access the effect of HPP or pH on egg white foaming properties. HPP significantly reduced foam stability with the exception of the 800 MPa 0 time point. This result was in contrast to another study (Van der Plancken et al 2007a), which found HP treatment increased overall foam stability. As foam stability is a function of liquid drainage from the foam, the increased stability of 800 MPa at the 0 time point was attributed to the increased foam volume and incorporation of liquid in the foam. However, liquid drainage was the greatest over the first 5 minutes post-foam (800 MPa) as indicated by the slope and drop in stability as seen in **Figure 29**.

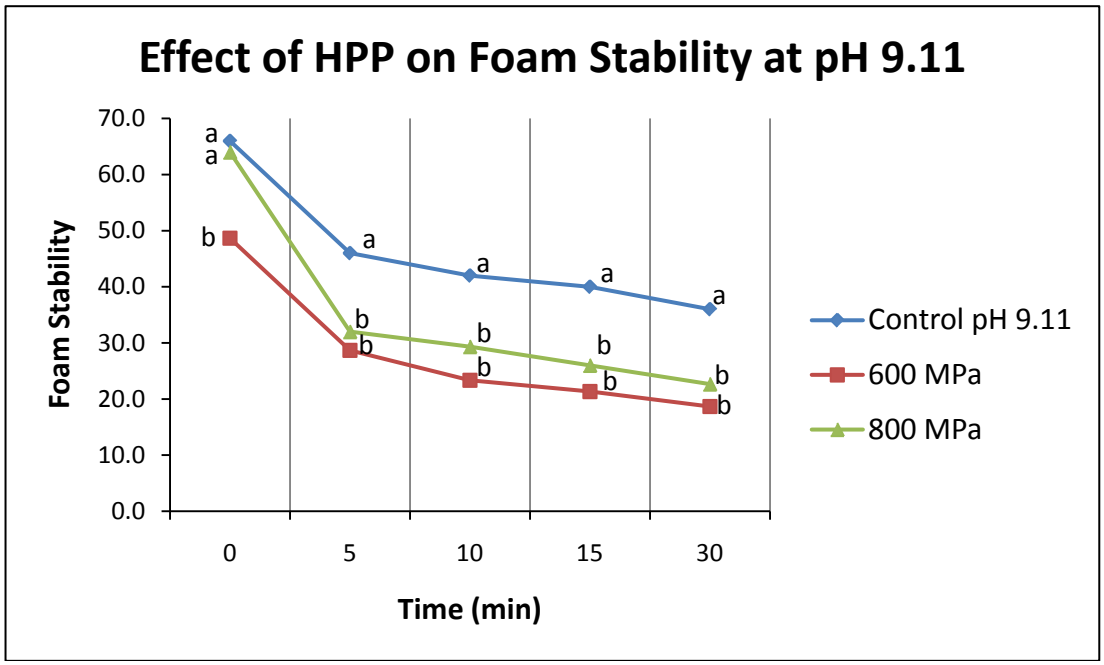

Figure 29: Effect of HPP (5 min) on 10% egg white solution foam stability at pH 9.11 and at 0.1 MPa (control), 600 MPa, and 800 MPa. Time intervals represent measurement of foam stability post-foaming. Same letters above each point for each time denote means are not significantly different (α=0.05) using Duncan's test and Dunnet least square means.

With respect to pH, egg white solutions at pH 4.5 were the most stable followed by the control sample at pH 9.11. The foam produced at pH 4.5 was also thicker and had a creamy texture. Foam stability was significantly decreased at pH 6. This was attributed to the volume of liquid not incorporated into the foam. The rate of foam collapse/liquid drainage at pH 6 was similar to control as seen in **Figure 30**.

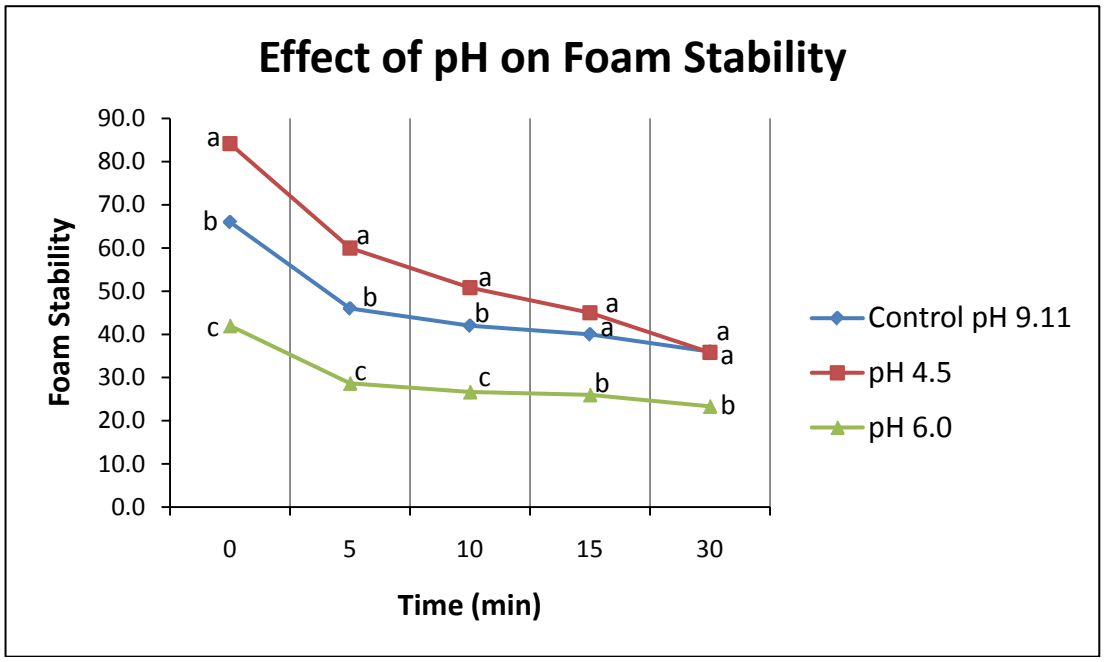

Figure 30: Effect of pH on 10% egg white solution foam stability at pH 9.11 (control), pH 6, and pH 4.5 at room temperature (20°C). Time intervals represent measurement of foam stability post-foaming. Same letters above each bar for each time denote means are not significantly different (α=0.05) using Duncan's test and Dunnet least square means.

Conclusions

The effects of HPP on egg white protein and its impact on egg white protein pepsin digestibility and egg white functional properties were evaluated. HPP increased digestibility of egg white proteins, specifically ovalbumin, lysozyme, and ovotransferrin as evidenced by SDS-PAGE. Two-dimensional electrophoretic analysis also indicated an increase in digestibility due to HPP. HPP resulted in a shift in secondary structure with an increase in β-sheet/α-helix ratio as determined via Raman analysis. The conformational changes in secondary structure indicate HPP promotes protein unfolding which may increase susceptibility to enzymatic hydrolysis. Another consequence of a shift in secondary structure may be a change in allergenicity of egg white proteins as some epitopes (ovalbumin) may be structurally dependant (Mine, Y. et al 2003). Sequential epitopes may also be fragmented leading to a reduction in IgE binding (Lopez-Exposito, I. et al 2008, Kovacs-Nolan, J. et al 2000). Similar effects on digestibility and secondary structure were also apparent with thermal treatments. Mass spectrometry analysis showed a relative abundance of peptide fragments isolated from 800 MPa egg white pepsin digestions compared to the control. ACE-inhibitory peptide YAEERYPIL was identified as a pepsin digestion product of HP-treated egg white. The evidence of greater protein fragmentation indicates HPP facilitates digestion of egg white proteins and possibly release of sequence embedded bioactive peptides. This work has shown the potential of HPP in increasing the overall nutrition of egg white (digestibility). HPP may also affect allergenicity by fragmentation of allergenic epitopes with changes in secondary structure.

Overall the effects of HPP on egg white functionality were favorable. HPP improved foaming properties of egg white at pressures up to 800 MPa. Due to precipitation of whole egg white proteins at pressures used in this study (400, 600, and 800 MPa), a combination of lower pressure and thermal treatment possibly could be applied to improve foaming properties of liquid egg products. Lowering the pH to 4.5 with potassium bitartrate resulted in the best foaming properties. HPP at pressures of 800 MPa resulted in firm egg white gels comparable to thermally-induced gels (95°). In general, HPP gels were less hard but more elastic than heat-treated gels. HPP gel texture properties were also more consistent. This was primarily due to uniform pressure application. Lowering the pH (pH 6) of egg white prior to HP or heat treatment improved overall gel appearance.

This work has presented some of the effects of HPP on egg white protein digestibility and functional properties. Egg white is one of the best sources of nutritional protein. Thus, more research is needed to evaluate the potential health benefits of HPP on egg white protein and the release of bioactive sequences. HPP is an emerging technology with the potential to increase nutritional benefits and add new functional properties to food products. As an alternative to thermal processes and HPP's inherent advantages discussed in this work, it is important to continue HPP research on food products.

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Appendix

Experimental Protocols

Raman Spectroscopy Protocol

Materials
Egg white

Sample Preparation
1. Measure pH of whole egg white.
2. Adjust to desired pH by adding granular tartaric acid to egg white
3. Place in flexible plastic sausage casing to from uniform cylinder upon treatment
4. Treat via thermal (95°C) or pressure treatment (400-800 MPa)
5. Place in VWR glass vials (#66011-020)

Raman Analysis: Enwave Optronics Spectrometer (785 nm laser)
1. Set integration time for 120s, average to 3, and boxcar smoothing to 2.
2. Measure spectrum at room temperature using untreated egg white as a control

In vitro digestibility (3:1 pepsin : protein ratio) Protocol

Solutions
Simulated Gastric Fluid (SGF): 0.1 N HCl, 0.03 M NaCl, pH 1.2 (Sigma G3285)
Pepsin: Crystallized Porcine 3000 units/mg (Sigma P6887)
0.2 M Na_2CO_3
10% SDS
0.4 M NH_4HCO_3

Pepsin Preparation
Dissolve 18 mg pepsin 10 mL of cold SGF. Vortex until completely dissolved. Keep on ice for 2 hours then discard

Sample Preparation
1. Determine protein concentration
2. Dilute egg white sample to a concentration of approximately 10 µg/µL (normally 1:10 dilution)

Digestion Procedure
1. Add 1.2 mL pepsin-SGF solution into a 1.5 mL microfuge tube and equilibrate at 37°C for 5 min. Pepsin solution should be freshly made and kept on ice. Discard after 2 hours.
2. Control: <u>0 time tube</u> : To this tube add 80 µL 0.2 M Na_2CO_3 and 10 µL 10% SDS followed by 50 µg test protein. Then add 200 µL of SGF-Pepsin and mix immediately
3. Digestion Reaction: Add an aliquot (max 70µL) of protein equivalent to 70 µg to the tube containing 1.2 mL pepsin-SGF. Timing of reaction starts from pipetting of protein.
4. Stop the reaction at 30 s, 2, 4, 8, 15, and 30 min by withdrawing 200 µL from the incubation tube and adding to a labeled tube containing 80 µL 0.2 M Na_2CO_3 and 10 µL 10 % SDS.
5. **<u>NOTE</u>:** Replace 80 µL 0.2 M Na_2CO_3 and 10 µL 10 % SDS solution with ~90 µL 0.4 M NH_4HCO_3 or enough to bring pH 7-8 for HPLC or LC/MS/MS samples.

For SDS-analysis:
1. Add 35 µL SDS sample prep solution and heat at 50°C for 2 min
2. Centrifuge for 2 min at 10,000 g and load 35 µL (~13µg protein) on 10-20% gradient gels or place in freezer at -20°C until SDS analysis.

For HPLC or LC/MS/MS:
1. Dry sample via centrivap and store at -20°C for analysis.

HPLC or LC/MS/MS sample preparation

1. Use only digestions stopped with 0.4 M NH_4HCO_3 stop solution
2. Centrifuge 10 min at 17,000 RCF and collect supernatant
3. Filter supernatant with YM3 3000 molecular cut off spin filters at 14,000 RCF until 10% of original volume remains (~30-45 minutes to filter 500 µL)
4. Dry using Centra-Vap and store at -20°C till analysis
5. For HPLC samples suspend in buffer and dilute accordingly for analysis
6. Purify samples for MS analysis with Pierce PepClean™ C-18 spin columns (#89873) and follow protocols as described in instruction manual.

Gelation Properties Protocol

Materials
Tartaric Acid (Acros Organics #137855000)
Egg white

Sample Preparation
6. Measure pH of whole egg white.
7. Adjust to desired pH by adding granular tartaric acid to egg white
8. Place in flexible plastic sausage casing to from uniform cylinder upon treatment
9. Induce gelation via thermal (95°C) or pressure treatment (600-800 MPa)
10. Carefully remove packaging making sure not to disturb gel structure

Textural Analysis: TA.XT2 Texture Analyzer
1. Calibrate texture analyzer with a 5 kg load cell
2. Cut egg gels to a height of 20 mm (diameter 23 mm)
3. Program texture analyzer to compress sample twice (2 bite cycle) with a 50 % penetration value and set compression speed to 1.2 mm/s
4. Fit the analyzer with cylindrical test probe TA-4, 37mm diameter and run cycle
5. Use Texture Technologies texture profile analysis to determine gel properties as described by Bourne (1982).

Color Analysis: Minolta colorimeter equipped with Hunter color difference meter
1. Cut egg samples to a height of 10 mm (diameter 23 mm)
2. Take color measurements using colorimeter (10°/D65 light source)

Foaming Abilities Protocol

Materials
0.2 % Potassium Bitartrate (KT) (Fisher Scientific S76971)
Egg white

Sample Preparation
11. Measure pH of whole egg white.
12. Control: 90 mL DI water, 10 mL egg white, measure and record pH
13. KT: Dilute egg white with 10-30 mL DI water before adjusting pH with KT solution. Adjust pH to 4.5 and pH 6 and bring final volume to 100 mL with DI water.

Approximate amounts of 0.2% KT solution for each pH with starting egg white pH of 9.1:

pH	Approximate mL0.2% KT
4	80-90 mL
5	60-70 mL
6	30-40 mL

Foaming
1. Separate 100 mL solutions of 10% egg white solutions into 30 mL aliquots in 400 mL beakers, making sure the solution is homogenous as possible before separation
2. Foam each 30 mL solution for 2 minutes
3. Transfer contents to graduated cylinder within 1:30 min
4. Give 2 quick shakes downward to settle foam in cylinder
5. Start timer and record total volume and volume of liquid
6. Record at 0, 5, 10, 15, and 30 minutes
7. Use these values to calculate volume of foam, foam overrun, and foam stability at each time point

$$Foam\ Overrun = \frac{foam\ volume}{30\ mL}$$

$$Foam\ Stablity = \frac{30\ mL - Liquid\ volume}{30\ mL} \times 100$$

$$Foam\ volume = Total\ Volume - Liquid\ Volume$$

A1: Peptide sequences of ovalbumin predicted by ExPASy Peptide Cutter with pepsin at pH 1.3.

Cleavage Site	Cleaving Enzyme	Resulting peptide sequence	Peptide length [aa]	Peptide mass [Da]
10	Pepsin(pH1.3)	MGSIGAASME	10	953.093
11	Pepsin (pH1.3)	F	1	165.192
12	Pepsin (pH1.3)	C	1	121.154
13	Pepsin (pH1.3)	F	1	165.192
15	Pepsin (pH1.3)	DV	2	232.236
16	Pepsin (pH1.3)	F	1	165.192
18	Pepsin (pH1.3)	KE	2	275.305
28	Pepsin (pH1.3)	LKVHHANENI	10	1174.325
29	Pepsin (pH1.3)	F	1	165.192
38	Pepsin (pH1.3)	YCPIAIMSA	9	968.194
39	Pepsin (pH1.3)	L	1	131.175
42	Pepsin (pH1.3)	AMV	3	319.419
43	Pepsin (pH1.3)	Y	1	181.191
44	Pepsin (pH1.3)	L	1	131.175
60	Pepsin (pH1.3)	GAKDSTRTQINKVVRF	16	1820.082
63	Pepsin (pH1.3)	DKL	3	374.437
66	Pepsin (pH1.3)	PGF	3	319.360
84	Pepsin (pH1.3)	GDSIEAQCGTSVNVHSSL	18	1803.918
88	Pepsin (pH1.3)	RDIL	4	515.610
97	Pepsin (pH1.3)	NQITKPNDV	9	1028.130
98	Pepsin (pH1.3)	Y	1	181.191
99	Pepsin (pH1.3)	S	1	105.093
100	Pepsin (pH1.3)	F	1	165.192
101	Pepsin (pH1.3)	S	1	105.093
102	Pepsin (pH1.3)	L	1	131.175
106	Pepsin (pH1.3)	ASRL	4	445.519
112	Pepsin (pH1.3)	YAEERY	6	829.865
115	Pepsin (pH1.3)	PIL	3	341.451
118	Pepsin (pH1.3)	PEY	3	407.423
119	Pepsin (pH1.3)	L	1	131.175
124	Pepsin (pH1.3)	QCVKE	5	605.707
126	Pepsin (pH1.3)	LY	2	294.351
134	Pepsin (pH1.3)	RGGLEPIN	8	854.961
135	Pepsin (pH1.3)	F	1	165.192
144	Pepsin (pH1.3)	QTAADQARE	9	989.010
148	Pepsin (pH1.3)	LINS	4	445.516
149	Pepsin (pH1.3)	W	1	204.228
174	Pepsin (pH1.3)	VESQTNGIIRNVLQPSSVDSQTAMV	25	2673.978
175	Pepsin (pH1.3)	L	1	131.175
180	Pepsin (pH1.3)	VNAIV	5	514.622

181	Pepsin (pH1.3)	F	1	165.192
183	Pepsin (pH1.3)	KG	2	203.241
185	Pepsin (pH1.3)	LW	2	317.388
188	Pepsin (pH1.3)	EKT	3	376.410
198	Pepsin (pH1.3)	FKDEDTQAMP	10	1181.283
212	Pepsin (pH1.3)	FRVTEQESKPVQMM	14	1709.996
213	Pepsin (pH1.3)	Y	1	181.191
216	Pepsin (pH1.3)	QIG	3	316.357
217	Pepsin (pH1.3)	L	1	131.175
218	Pepsin (pH1.3)	F	1	165.192
230	Pepsin (pH1.3)	RVASMASEKMKI	12	1350.658
233	Pepsin (pH1.3)	LEL	3	373.450
234	Pepsin (pH1.3)	P	1	115.132
242	Pepsin (pH1.3)	FASGTMSM	8	830.969
243	Pepsin (pH1.3)	L	1	131.175
244	Pepsin (pH1.3)	V	1	117.148
246	Pepsin (pH1.3)	LL	2	244.334
252	Pepsin (pH1.3)	PDEVSG	6	602.599
253	Pepsin (pH1.3)	L	1	131.175
255	Pepsin (pH1.3)	EQ	2	275.261
256	Pepsin (pH1.3)	L	1	131.175
261	Pepsin (pH1.3)	ESIIN	5	574.632
262	Pepsin (pH1.3)	F	1	165.192
264	Pepsin (pH1.3)	EK	2	275.305
265	Pepsin (pH1.3)	L	1	131.175
267	Pepsin (pH1.3)	TE	2	248.236
268	Pepsin (pH1.3)	W	1	204.228
281	Pepsin (pH1.3)	TSSNVMEERKIKV	13	1520.764
283	Pepsin (pH1.3)	YL	2	294.351
291	Pepsin (pH1.3)	PRMKMEEK	8	1048.284
292	Pepsin (pH1.3)	Y	1	181.191
294	Pepsin (pH1.3)	NL	2	245.278
297	Pepsin (pH1.3)	TSV	3	305.331
298	Pepsin (pH1.3)	L	1	131.175
306	Pepsin (pH1.3)	MAMGITDV	8	837.017
307	Pepsin (pH1.3)	F	1	165.192
312	Pepsin (pH1.3)	SSSAN	5	464.432
313	Pepsin (pH1.3)	L	1	131.175
321	Pepsin (pH1.3)	SGISSAES	8	736.734
322	Pepsin (pH1.3)	L	1	131.175
358	Pepsin (pH1.3)	KISQAVHAAHAEINEAGRE VVGSAEAGVDAASVSEE	36	3589.835
359	Pepsin (pH1.3)	F	1	165.192
364	Pepsin (pH1.3)	RADHP	5	594.628
366	Pepsin (pH1.3)	FL	2	278.351

367	Pepsin (pH1.3)	F	1	165.192
377	Pepsin (pH1.3)	CIKHIATNAV	10	1069.287
378	Pepsin (pH1.3)	L	1	131.175
379	Pepsin (pH1.3)	F	1	165.192
380	Pepsin (pH1.3)	F	1	165.192
386	end of sequence	GRCVSP	6	617.721

A2: Proteins identified via mass spectrometry after 2-D electrophoresis. Included in the table are peptide fragments identified and corresponding mouse scores.

Spot #	Protein Indentification	Gi	Peptides Matches					
1	Chain A, Crystal Structure Of S-Ovalbumin	gi|34811330	34	Observed	Mr(expt)	Mr(calc)	Score	Peptide
				390.72	779.42	779.38	40	R.LYAEER.Y
				605.28	1208.54	1208.51	61	K.DEDTQAMPFR.V
				624.34	1246.67	1246.62	31	R.ADHPFLFCIK.H
				673.39	1344.77	1344.73	83	K.HIATNAVLFFGR.C
				778.38	1554.74	1554.71	90	K.AFKDEDTQAMPFR.V
				791.38	1580.74	1580.71	112	K.LTEWTSSNVMEER.K
				844.4	1686.78	1686.83	93	R.GGLEPINFQTAADQAR.E
				592	1772.97	1772.89	99	K.ISQAVHAAHAEINEAGR.E
				761.02	2280.05	2280.17	95	R.DILNQITKPNDVYSFSLASR.L
				767.4	2299.19	2299.13	70	R.VTEQESKPVQMMYQIGLFR.V + Oxidation (M)
				1246.69	2491.36	2491.3	115	R.NVLQPSSVDSQTAMVLVNAIVFK.G + Dioxidation (M)
				1022.54	3064.58	3064.5	42	K.VHHANENIFYCPIAIMSALAMVYLGAK.D + 2 Oxidation (M)
				1030.57	3088.7	3088.63	41	R.NVLQPSSVDSQTAMVLVNAIVFKGLWEK.A + Oxidation (M)
				1299.04	3894.09	3893.99	114	K.ILELPFAAGTMSMLVLLPDEVSGLEQLESIINFEK.L + Dioxidation (M); Oxidation (M)
1	Ovomucoid	gi|124757	5	Observed	Mr(expt)	Mr(calc)	Score	Peptide
				446.27	890.53	890.45	39	K.VMVLCNR.A
				544.8	1087.59	1087.56	76	K.VEQGASVDKR.H
				881.04	2640.1	2640.14	86	R.AFNPVCGTDGVTYDNECLLCAHK.V
1	ovalbumin-related protein Y	gi|71897377	1	Observed	Mr(expt)	Mr(calc)	Score	Peptide
				533.78	1065.54	1065.51	58	K.IAFNTEDTR.E
2	Chain A, Crystal Structure Of S-Ovalbumin	gi|34811330	10	Observed	Mr(expt)	Mr(calc)	Score	Peptide
				799.39	1596.76	1596.71	75	K.LTEWTSSNVMEER.K + Oxidation (M)
				844.43	1686.85	1686.83	96	R.GGLEPINFQTAADQAR.E
				772.74	2315.2	2315.13	31	R.VTEQESKPVQMMYQIGLFR.V + 2 Oxidation (M)
				826.14	2475.38	2475.3	87	R.NVLQPSSVDSQTAMVLVNAIVFK.G + Oxidation (M)
				761.41	3041.62	3041.55	1	R.DILNQITKPNDVYSFSLASRLYAEER.Y
				1022.53	3064.58	3064.5	27	K.VHHANENIFYCPIAIMSALAMVYLGAK.D + 2 Oxidation (M)
2	Ovomucoid	gi|124757	12	Observed	Mr(expt)	Mr(calc)	Score	Peptide
				446.27	890.53	890.45	38	K.VMVLCNR.A
				881.09	2640.26	2640.14	68	R.AFNPVCGTDGVTYDNECLLCAHK.V
2	Ovotransferrin	gi|1351295	1					
				Observed	Mr(expt)	Mr(calc)	Score	Peptide
				534.27	1066.52	1066.49	53	K.AQSDFGVDTK.S
3	Chain A, Crystal Structure Of S-Ovalbumin	gi|34811330	43	Observed	Mr(expt)	Mr(calc)	Score	Peptide
				390.72	779.42	779.38	48	R.LYAEER.Y
				613.27	1224.53	1224.51	38	K.DEDTQAMPFR.V + Oxidation (M)
				624.34	1246.67	1246.62	37	R.ADHPFLFCIK.H
				749.41	1496.81	1496.76	2	R.YPILPEYLQCVK.E + Dioxidation (C)
				778.39	1554.77	1554.71	82	K.AFKDEDTQAMPFR.V
				791.36	1580.7	1580.71	112	K.LTEWTSSNVMEER.K
				794.4	1586.78	1586.7	-7	K.AFKDEDTQAMPFR.V + Dioxidation (M)
				807.38	1612.75	1612.7	-28	K.LTEWTSSNVMEER.K + Dioxidation (M)
				844.38	1686.74	1686.83	96	R.GGLEPINFQTAADQAR.E
				591.99	1772.95	1772.89	92	K.ISQAVHAAHAEINEAGR.E
				761.1	2280.28	2280.17	74	R.DILNQITKPNDVYSFSLASR.L
				772.71	2315.12	2315.13	61	R.VTEQESKPVQMMYQIGLFR.V + 2 Oxidation (M)
				1230.71	2459.4	2459.31	91	R.NVLQPSSVDSQTAMVLVNAIVFK.G
				1049.59	3145.74	3145.65	1	R.NVLQPQSVDSQTAMVLVNAIVFKGLWEK.A + Dioxidation (M)
				1299.05	3894.12	3893.99	99	K.ILELPFAAGTMSMLVLLPDEVSGLEQLESIINFEK.L + Dioxidation (M); Oxidation (M)

3	ovalbumin	gi|28566340	39	Observed	Mr(expt)	Mr(calc)	Score	Peptide
				390.72	779.42	779.38	48	R.LYAEER.Y
				613.27	1224.53	1224.51	38	K.DEDTQAMPFR.V + Oxidation (M)
				624.34	1246.67	1246.62	37	R.ADHPFLFCIK.H
				749.41	1496.81	1496.76	2	R.YPILPEYLQCVK.E + Dioxidation (C)
				778.39	1554.77	1554.71	82	K.AFKDEDTQAMPFR.V
				791.36	1580.7	1580.71	112	K.LTEWTSSNVMEER.K
				844.38	1686.74	1686.83	96	R.GGLEPINFQTAADQAR.E
				591.99	1772.95	1772.89	92	K.ISQAVHAAHAEINEAGR.E
				761.1	2280.28	2280.17	74	R.DILNQITKPNDVYSFSLASR.L
				772.71	2315.12	2315.13	61	R.VTEQESKPVQMMYQIGLFR.V + 2 Oxidation (M)
				1230.71	2459.4	2459.31	91	R.NVLQPSSVDSQTAMVLVNAIVFK.G
				1299.05	3894.12	3893.99	99	K.ILELPFASGTMSMLVLLPDEVSGLEQLESIINFEK.L + Dioxidation (M)
3	Ovalbumin-related protein X	gi|129295	2	Observed	Mr(expt)	Mr(calc)	Score	Peptide
				512.76	1023.5	1023.46	63	K.TAFNAEDTR.E
				963.16	2886.45	2886.49	49	K.ILELPFASGDLSMLVLLPDEVSDLER.I + Oxidation (M)
3	ovalbumin-related protein Y	gi|71897377	1	Observed	Mr(expt)	Mr(calc)	Score	Peptide
				533.78	1065.54	1065.51	58	K.IAFNTEDTR.E
4	ovalbumin-related protein Y	gi|71897377	3	Observed	Mr(expt)	Mr(calc)	Score	Peptide
				676.82	1351.62	1351.56	54	K.FCFDVFNEMK.V
				695.38	1388.74	1388.66	30	K.FYTGGVEEVNFK.T
				771.91	1541.81	1541.75	47	K.TFSVLPEYLSCAR.K
5	Chain A, Crystal Structure Of S-Ovalbumin	gi|34811330	44	Observed	Mr(expt)	Mr(calc)	Score	Peptide
				605.29	1208.57	1208.51	66	K.DEDTQAMPFR.V
				778.4	1554.78	1554.71	75	K.AFKDEDTQAMPFR.V
				791.38	1580.74	1580.71	98	K.LTEWTSSNVMEER.K
				844.47	1686.92	1686.83	99	R.GGLEPINFQTAADQAR.E
				592.01	1773.01	1772.89	76	K.ISQAVHAAHAEINEAGR.E
				620.35	1858.03	1857.96	50	R.ELINSWVESQTNGIIR.N
				750.42	2248.23	2248.12	52	K.ELYRGGLEPINFQTAADQAR.E
				772.74	2315.2	2315.13	63	R.VTEQESKPVQMMYQIGLFR.V + 2 Oxidation (M)
				831.47	2491.4	2491.3	97	R.NVLQPSSVDSQTAMVLVNAIVFK.G + Dioxidation (M)
				1030.6	3088.77	3088.63	66	R.NVLQPSSVDSQTAMVLVNAIVFKGLWEK.A + Oxidation (M)
5	ovalbumin	gi|28566340	41	Observed	Mr(expt)	Mr(calc)	Score	Peptide
				605.29	1208.57	1208.51	66	K.DEDTQAMPFR.V
				778.4	1554.78	1554.71	75	K.AFKDEDTQAMPFR.V
				791.38	1580.74	1580.71	98	K.LTEWTSSNVMEER.K
				844.47	1686.92	1686.83	99	R.GGLEPINFQTAADQAR.E
				592.01	1773.01	1772.89	76	K.ISQAVHAAHAEINEAGR.E
				620.35	1858.03	1857.96	50	R.ELINSWVESQTNGIIR.N
				750.42	2248.23	2248.12	52	K.ELYRGGLEPINFQTAADQAR.E
				772.74	2315.2	2315.13	63	R.VTEQESKPVQMMYQIGLFR.V + 2 Oxidation (M)
				831.47	2491.4	2491.3	97	R.NVLQPSSVDSQTAMVLVNAIVFK.G + Dioxidation (M)
				1030.6	3088.77	3088.63	66	R.NVLQPSSVDSQTAMVLVNAIVFKGLWEK.A + Oxidation (M)
5	unnamed protein product [Gallus gallus]	gi|63052	12	Observed	Mr(expt)	Mr(calc)	Score	Peptide
				710.38	1418.75	1418.67	25	R.ELINSWVESQTN.-
				844.47	1686.92	1686.83	99	R.GGLEPINFQTAADQAR.E
				750.42	2248.23	2248.12	52	K.ELYRGGLEPINFQTAADQAR.E
6	Chain A, Crystal Structure Of S-Ovalbumin	gi|34811330	21	Observed	Mr(expt)	Mr(calc)	Score	Peptide
				613.28	1224.55	1224.51	57	K.DEDTQAMPFR.V + Oxidation (M)
				673.4	1344.78	1344.73	69	K.HIATNAVLFFGR.C
				786.39	1570.77	1570.71	47	K.AFKDEDTQAMPFR.V + Oxidation (M)
				791.39	1580.76	1580.71	112	K.LTEWTSSNVMEER.K
				844.45	1686.89	1686.83	44	R.GGLEPINFQTAADQAR.E
				887.49	1772.96	1772.89	76	K.ISQAVHAAHAEINEAGR.E
				620.35	1858.03	1857.96	72	R.ELINSWVESQTNGIIR.N
				772.73	2315.17	2315.13	69	R.VTEQESKPVQMMYQIGLFR.V + 2 Oxidation (M)
				826.14	2475.4	2475.3	76	R.NVLQPSSVDSQTAMVLVNAIVFK.G + Oxidation (M)
				1019.5	3055.48	3055.46	60	K.VHHANENIFYCPIAIMSALAMVYLGAK.D + Dioxidation (M); Oxidation (M); Dioxidation (C)
				1299.05	3894.12	3893.99	102	K.ILELPFASGTMSMLVLLPDEVSGLEQLESIINFEK.L + 2 Oxidation (M)
6	protein TENP [Gallus gallus]	gi|46048814	4	Observed	Mr(expt)	Mr(calc)	Score	Peptide
				820	1637.98	1637.91	70	K.VADLWLSVIPEAGLR.L
				766.42	2296.23	2296.24	73	K.ITQVGSLYHEDLPITLSAALR.S
				967.53	2899.55	2899.46	49	R.AALLEELFLAPVCQQVPAWMDDVLR.E + Oxidation (M)
				817.67	3266.66	3266.58	46	R.ADLHVDMGPDGNLQLLTSACRPTVQAQSTR.E +

								Oxidation (M)
6	Ovotransferrin	gi|1351295	3	Observed	Mr(expt)	Mr(calc)	Score	Peptide
				481.26	960.51	960.49	38	R.IQWCAVGK.D
				521.31	1040.61	1040.56	60	R.KDQLTPSPR.E
				885.12	2652.34	2652.24	88	R.WSVVSNGDVECTVVDETKDCIIK.I
6	Chain A,Chicken Egg-White Lysozyme Core Mutants	gi|157831883	1	Observed	Mr(expt)	Mr(calc)	Score	Peptide
				877.45	1752.89	1752.83	106	R.NTDGSTDYGLLQINSR.W
6	ovalbumin-related protein Y	gi|71897377	2	Observed	Mr(expt)	Mr(calc)	Score	Peptide
				501.74	1001.47	1001.42	23	R.EMPFSMTK.E + 2 Oxidation (M)
				706.89	1411.76	1411.72	47	R.YNPTNAILFFGR.Y
6	Ovalbumin-related protein X	gi|129295	1	Observed	Mr(expt)	Mr(calc)	Score	Peptide
				512.76	1023.51	1023.46	67	K.TAFNAEDTR.E
7	Chain A, Crystal Structure Of S-Ovalbumin	gi|34811330	7	Observed	Mr(expt)	Mr(calc)	Score	Peptide
				799.39	1596.78	1596.71	70	K.LTEWTSSNVMEER.K + Oxidation (M)
				844.46	1686.9	1686.83	90	R.GGLEPINFQTAADQAR.E
				772.74	2315.2	2315.13	22	R.VTEQESKPVQMMYQIGLFR.V + 2 Oxidation (M)
				826.13	2475.37	2475.3	92	R.NVLQPSSVDSQTAMVLVNAIVFK.G + Oxidation (M)
				1299.03	3894.08	3893.99	88	K.ILELPFAAGTMSMLVLLPDEVSGLEQLESIINFEK.L + Dioxidation (M); Oxidation (M)
7	Ovalbumin	gi|129293	7	Observed	Mr(expt)	Mr(calc)	Score	Peptide
				799.39	1596.78	1596.71	70	K.LTEWTSSNVMEER.K + Oxidation (M)
				844.46	1686.9	1686.83	90	R.GGLEPINFQTAADQAR.E
				772.74	2315.2	2315.13	22	R.VTEQESKPVQMMYQIGLFR.V + 2 Oxidation (M)
				826.13	2475.37	2475.3	92	R.NVLQPSSVDSQTAMVLVNAIVFK.G + Oxidation (M)
				1299.03	3894.08	3893.99	86	K.ILELPFASGTMSMLVLLPDEVSGLEQLESIINFEK.L + Dioxidation (M)
8	Ovalbumin	gi|129293	7	Observed	Mr(expt)	Mr(calc)	Score	Peptide
				791.38	1580.75	1580.71	108	K.LTEWTSSNVMEER.K
				844.44	1686.86	1686.83	90	R.GGLEPINFQTAADQAR.E
				891.42	1780.82	1780.78	30	M.GSIGAASMEFCFDVFK.E + Oxidation (M)
				826.13	2475.36	2475.3	94	R.NVLQPSSVDSQTAMVLVNAIVFK.G + Oxidation (M)
				1298.99	3893.93	3893.99	69	K.ILELPFASGTMSMLVLLPDEVSGLEQLESIINFEK.L + Dioxidation (M)
8	ovalbumin N term fragment	gi|223059	5	Observed	Mr(expt)	Mr(calc)	Score	Peptide
				891.42	1780.82	1780.78	30	-.GSIGAASMEFCFDVFK.E + Oxidation (M)
				734.05	2199.12	2199.06	41	K.VHHANENIFYCPIAIMSAL.-
8	ovalbumin-related protein Y	gi|71897377	1	Observed	Mr(expt)	Mr(calc)	Score	Peptide
				676.79	1351.56	1351.56	62	K.FCFDVFNEMK.V + Oxidation (M)
9	Chain A, Crystal Structure Of S-Ovalbumin	gi|34811330	16	Observed	Mr(expt)	Mr(calc)	Score	Peptide
				605.28	1208.54	1208.51	66	K.DEDTQAMPFR.V
				778.36	1554.7	1554.71	96	K.AFKDEDTQAMPFR.V
				791.38	1580.75	1580.71	85	K.LTEWTSSNVMEER.K
				772.72	2315.14	2315.13	74	R.VTEQESKPVQMMYQIGLFR.V + 2 Oxidation (M)
				826.12	2475.33	2475.3	95	R.NVLQPSSVDSQTAMVLVNAIVFK.G + Oxidation (M)
9	Ovalbumin	gi|129295	1	Observed	Mr(expt)	Mr(calc)	Score	Peptide
				512.75	1023.48	1023.46	63	K.TAFNAEDTR.E
9	ovalbumin-related protein Y	gi|71897377	1	Observed	Mr(expt)	Mr(calc)	Score	Peptide
				676.79	1351.57	1351.56	49	K.FCFDVFNEMK.V + Oxidation (M)
10	Ovalbumin	gi|129293	31	Observed	Mr(expt)	Mr(calc)	Score	Peptide
				624.31	1246.6	1246.62	49	R.ADHPFLFCIK.H
				673.38	1344.74	1344.73	79	K.HIATNAVLFFGR.C
				524.59	1570.74	1570.71	72	K.AFKDEDTQAMPFR.V + Oxidation (M)
				791.38	1580.74	1580.71	108	K.LTEWTSSNVMEER.K
				844.43	1686.84	1686.83	58	R.GGLEPINFQTAADQAR.E
				887.42	1772.83	1772.89	129	K.ISQAVHAAHAEINEAGR.E
				891.42	1780.83	1780.78	37	M.GSIGAASMEFCFDVFK.E + Oxidation (M)
				1005	2007.98	2007.94	147	R.EVVGSAEAGVDAASVSEEFR.A
				772.72	2315.14	2315.13	62	R.VTEQESKPVQMMYQIGLFR.V + 2 Oxidation (M)
				826.1	2475.29	2475.3	93	R.NVLQPSSVDSQTAMVLVNAIVFK.G + Oxidation (M)
				1022.52	3064.54	3064.5	80	K.VHHANENIFYCPIAIMSALAMVYLGAK.D + 2 Oxidation (M)
				1299.03	3894.06	3893.99	40	K.ILELPFASGTMSMLVLLPDEVSGLEQLESIINFEK.L + Dioxidation (M)

10	Chain A, Crystal Structure Of S-Ovalbumin	gi|34811330	31	Observed	Mr(expt)	Mr(calc)	Score	Peptide
				624.31	1246.6	1246.62	49	R.ADHPFLFCIK.H
				673.38	1344.74	1344.73	79	K.HIATNAVLFFGR.C
				524.59	1570.74	1570.71	72	K.AFKDEDTQAMPFR.V + Oxidation (M)
				791.38	1580.74	1580.71	108	K.LTEWTSSNVMEER.K
				844.43	1686.84	1686.83	58	R.GGLEPINFQTAADQAR.E
				887.42	1772.83	1772.89	129	K.ISQAVHAAHAEINEAGR.E
				891.42	1780.83	1780.78	37	-.GSIGAASMEFCFDVFK.E + Oxidation (M)
				1005	2007.98	2007.94	147	R.EVVGSAEAGVDAASVSEEFR.A
				772.72	2315.14	2315.13	62	R.VTEQESKPVQMMYQIGLFR.V + 2 Oxidation (M)
				826.1	2475.29	2475.3	93	R.NVLQPSSVDSQTAMVLVNAIVFK.G + Oxidation (M)
				1022.52	3064.54	3064.5	80	K.VHHANENIFYCPIAIMSALAMVYLGAK.D + 2 Oxidation (M)
				1299.03	3894.06	3893.99	40	K.ILELPFAAGTMSMLVLLPDEVSGLEQLESIINFEK.L + Dioxidation (M); Oxidation (M)
10	Ovotransferrin	gi|1351295	6	Observed	Mr(expt)	Mr(calc)	Score	Peptide
				521.29	1040.57	1040.56	60	R.KDQLTPSPR.E
				524.27	1046.52	1046.52	42	K.YFGYTGALR.C
				534.26	1066.51	1066.49	50	K.AQSDFGVDTK.S
				445.24	1332.7	1332.65	21	R.IQWCAVGKDEK.S
				512.32	1533.94	1533.84	73	R.SAGWNIPIGTLLHR.G
				848.42	1694.84	1694.82	38	R.DDNKVEDIWSFLSK.A
10	ovotransferrin BB type	gi|71274075	6	Observed	Mr(expt)	Mr(calc)	Score	Peptide
				521.29	1040.57	1040.56	60	R.KDQLTPSPR.E
				524.27	1046.52	1046.52	42	K.YFGYTGALR.C
				534.26	1066.51	1066.49	50	K.AQSDFGVDTK.S
				445.24	1332.7	1332.65	21	R.IQWCAVGKDEK.S
				512.32	1533.94	1533.84	73	R.SAGWNIPIGTLIHR.G
				848.42	1694.84	1694.82	38	R.DDNKVEDIWSFLSK.A
10	Ovalbumin	gi|129294	10	Observed	Mr(expt)	Mr(calc)	Score	Peptide
				887.42	1772.83	1772.89	129	K.ISQAVHAAHAEINEAGR.D
				891.42	1780.83	1780.78	37	M.GSIGAASMEFCFDVFK.E + Oxidation (M)
				558.28	2229.11	2229.08	14	R.VTEQESKPVQMMYQIGSFK.V
10	protein TENP [Gallus gallus]	gi|46048814	4	Observed	Mr(expt)	Mr(calc)	Score	Peptide
				819.98	1637.94	1637.91	49	K.VADLWLSVIPEAGLR.L
				967.51	2899.5	2899.46	64	R.AALLEELFLAPVCQQVPAWMDDVLR.E + Oxidation (M)
				817.65	3266.56	3266.58	54	R.ADLHVDMGPDGNLQLLTSACRPTVQAQSTR.E + Oxidation (M)
10	ovoinhibitor precursor	gi|71895337	2	Observed	Mr(expt)	Mr(calc)	Score	Peptide
				574.78	1147.54	1147.51	54	K.DGTSWVACPR.N
				926.76	2777.26	2777.22	86	R.NLKPVCGTDGSTYSNECGICLYNR.E
10	Chain A, Chicken Egg-White Lysozyme Core Mutants	gi|157831883	1	Observed	Mr(expt)	Mr(calc)	Score	Peptide
				877.44	1752.87	1752.83	110	R.NTDGSTDYGLLQINSR.W
11	Ovalbumin	gi|129293	29	Observed	Mr(expt)	Mr(calc)	Score	Peptide
				382.28	762.54	762.44	21	K.VVRFDK.L
				605.28	1208.54	1208.51	62	K.DEDTQAMPFR.V
				624.33	1246.65	1246.62	37	R.ADHPFLFCIK.H
				673.38	1344.75	1344.73	65	K.HIATNAVLFFGR.C
				778.38	1554.75	1554.71	91	K.AFKDEDTQAMPFR.V
				791.38	1580.75	1580.71	118	K.LTEWTSSNVMEER.K
				844.42	1686.83	1686.83	93	R.GGLEPINFQTAADQAR.E
				591.99	1772.95	1772.89	73	K.ISQAVHAAHAEINEAGR.E
				761.04	2280.1	2280.17	95	R.DILNQITKPNDVYSFSLASR.L
				772.7	2315.09	2315.13	71	R.VTEQESKPVQMMYQIGLFR.V + 2 Oxidation (M)
				826.1	2475.28	2475.3	102	R.NVLQPSSVDSQTAMVLVNAIVFK.G + Oxidation (M)
				1022.52	3064.53	3064.5	123	K.VHHANENIFYCPIAIMSALAMVYLGAK.D + 2 Oxidation (M)
11	Ovotransferrin	gi|1351295	10	Observed	Mr(expt)	Mr(calc)	Score	Peptide
				481.26	960.51	960.49	42	R.IQWCAVGK.D
				521.29	1040.56	1040.56	52	R.KDQLTPSPR.E
				524.27	1046.52	1046.52	39	K.YFGYTGALR.C
				534.26	1066.51	1066.49	50	K.AQSDFGVDTK.S
				601.23	1200.45	1200.59	26	K.SDFHLFGPPGK.K
				667.35	1332.68	1332.65	56	R.IQWCAVGKDEK.S
				718.88	1435.74	1435.73	35	K.KGTEFTVNDLQGK.T
				735.36	1468.71	1468.65	49	K.DEYELLCLDGSR.Q
				885.1	2652.29	2652.24	96	R.WSVVSNGDVECTVVDETKDCIIK.I
11	unnamed protein product [Gallus gallus]	gi|63052	17	Observed	Mr(expt)	Mr(calc)	Score	Peptide
				382.28	762.54	762.44	21	K.VVRFDK.L
				844.42	1686.83	1686.83	93	R.GGLEPINFQTAADQAR.E
				877.9	1753.78	1753.77	12	M.GSIAAASMEFCFDVFK.E + Oxidation (M);

								Oxidation (C)
				761.04	2280.1	2280.17	95	R.DILNQITKPNDVYSFSLASR.L
				1022.52	3064.53	3064.5	123	K.VHHANENIFYCPIAIMSALAMVYLGAK.D + 2 Oxidation (M)
11	protein TENP [Gallus gallus]	gi|46048814	3	Observed	Mr(expt)	Mr(calc)	Score	Peptide
				819.97	1637.92	1637.91	84	K.VADLWLSVIPEAGLR.L
				967.51	2899.5	2899.46	54	R.AALLEELFLAPVCQQVPAWMDDVLR.E + Oxidation (M)
				817.68	3266.68	3266.58	74	R.ADLHVDMGPDGNLQLLTSACRPTVQAQSTR.E + Oxidation (M)
11	Ovalbumin	gi|129294	2	Observed	Mr(expt)	Mr(calc)	Score	Peptide
				472.77	943.53	943.53	17	R.DILNQITK.Q
				591.99	1772.95	1772.89	73	K.ISQAVHAAHAEINEAGR.D
11	ovalbumin N term fragment	gi|223059	3	Observed	Mr(expt)	Mr(calc)	Score	Peptide
				739.37	2215.09	2215.06	55	K.VHHANENIFYCPIAIMAAL.- + Dioxidation (M)
11	ovalbumin-related protein Y	gi|71897377	2	Observed	Mr(expt)	Mr(calc)	Score	Peptide
				533.77	1065.52	1065.51	55	K.IAFNTEDTR.E
				676.8	1351.59	1351.56	17	K.FCFDVFNEMK.V + Oxidation (M)
12	Ovotransferrin	gi|1351295	17	Observed	Mr(expt)	Mr(calc)	Score	Peptide
				404.19	806.37	806.35	27	K.TCNWAR.V
				444.22	886.43	886.41	19	K.SCHTAVGR.T
				474.77	947.52	947.51	38	R.ISLTCVQK.A
				481.25	960.48	960.49	34	R.IQWCAVGK.D
				494.76	987.5	987.46	55	K.TSCHTGLGR.S
				521.29	1040.56	1040.56	60	R.KDQLTPSPR.E
				524.27	1046.53	1046.52	44	K.YFGYTGALR.C
				534.26	1066.51	1066.49	53	K.AQSDFGVDTK.S
				612.32	1222.62	1222.62	54	K.VEDIWSFLSK.A
				667.34	1332.66	1332.65	65	R.IQWCAVGKDEK.S
				718.87	1435.73	1435.73	135	K.KGTEFTVNDLQGK.T
				512.27	1533.77	1533.84	72	R.SAGWNIPIGTLLHR.G
				822.42	1642.83	1642.78	84	R.LCQLCQGSGGIPPEK.C
				848.42	1694.83	1694.82	79	R.DDNKVEDIWSFLSK.A
				980.5	1958.99	1958.96	74	R.GAIEWEGIESGSVEQAVAK.F
				1012.48	2022.94	2022.92	94	R.WSVVSNGDVECTVVDETK.D
12	ovotransferrin BB type	gi|71274075	17	Observed	Mr(expt)	Mr(calc)	Score	Peptide
				404.19	806.37	806.35	27	K.TCNWAR.V
				444.22	886.43	886.41	19	K.SCHTAVGR.T
				474.77	947.52	947.51	38	R.ISLTCVQK.A
				481.25	960.48	960.49	34	R.IQWCAVGK.D
				494.76	987.5	987.46	55	K.TSCHTGLGR.S
				521.29	1040.56	1040.56	60	R.KDQLTPSPR.E
				524.27	1046.53	1046.52	44	K.YFGYTGALR.C
				534.26	1066.51	1066.49	53	K.AQSDFGVDTK.S
				612.32	1222.62	1222.62	54	K.VEDIWSFLSK.A
				667.34	1332.66	1332.65	65	R.IQWCAVGKDEK.S
				718.87	1435.73	1435.73	135	K.KGTEFTVNDLQGK.T
				512.27	1533.77	1533.84	72	R.SAGWNIPIGTLIHR.G
				822.42	1642.83	1642.78	84	R.LCQLCQGSGGIPPEK.C
				848.42	1694.83	1694.82	79	R.DDNKVEDIWSFLSK.A
				980.5	1958.99	1958.96	74	R.GAIEWEGIESGSVEQAVAK.F
				1012.48	2022.94	2022.92	94	R.WSVVSNGDVECTVVDETK.D
12	Ovalbumin	gi|129293	12	Observed	Mr(expt)	Mr(calc)	Score	Peptide
				524.59	1570.74	1570.71	77	K.AFKDEDTQAMPFR.V + Oxidation (M)
				891.43	1780.85	1780.78	11	M.GSIGAASMEFCFDVFK.E + Oxidation (M)
				772.73	2315.16	2315.13	46	R.VTEQESKPVQMMYQIGLFR.V + 2 Oxidation (M)
				826.13	2475.35	2475.3	82	R.NVLQPSSVDSQTAMVLVNAIVFK.G + Oxidation (M)
				1022.52	3064.53	3064.5	103	K.VHHANENIFYCPIAIMSALAMVYLGAK.D + 2 Oxidation (M)
12	Ovotransferrin	gi|3024757	8	Observed	Mr(expt)	Mr(calc)	Score	Peptide
				404.19	806.37	806.35	27	K.TCNWAR.V
				444.22	886.43	886.41	19	K.SCHTAVGR.T
				481.25	960.48	960.49	34	K.IQWCAVGK.D
				494.76	987.5	987.46	55	K.TSCHTGLGR.S
				667.34	1332.66	1332.65	65	K.IQWCAVGKDEK.S
				512.27	1533.77	1533.84	72	R.SAGWNIPIGTLIHR.E
				1012.48	2022.94	2022.92	10	R.WSVVSNGEVECTILDDNK.D + Oxidation (C)
12	Lysozyme	gi|126608	2	Observed	Mr(expt)	Mr(calc)	Score	Peptide
				714.85	1427.68	1427.64	92	K.FESNFNTQATNR.N
				877.43	1752.85	1752.83	126	R.NTDGSTDYGILQINSR.W
12	Chain A, Chicken Egg-White Lysozyme Core Mutants	gi|157831883	2	Observed	Mr(expt)	Mr(calc)	Score	Peptide
				714.85	1427.68	1427.64	92	K.FESNFNTQATNR.N
				877.43	1752.85	1752.83	126	R.NTDGSTDYGLLQINSR.W
12	Ovomucoid	gi|124757	4	Observed	Mr(expt)	Mr(calc)	Score	Peptide

				544.8	1087.59	1087.56	75	K.VEQGASVDKR.H
				881.02	2640.05	2640.14	87	R.AFNPVCGTDGVTYDNECLLCAHK.V
12	protein TENP [Gallus gallus]	gi|46048814	1	Observed	Mr(expt)	Mr(calc)	Score	Peptide
				819.97	1637.93	1637.91	100	K.VADLWLSVIPEAGLR.L
13	Ovalbumin	gi|129293	12	Observed	Mr(expt)	Mr(calc)	Score	Peptide
				613.27	1224.52	1224.51	54	K.DEDTQAMPFR.V + Oxidation (M)
				524.59	1570.74	1570.71	75	K.AFKDEDTQAMPFR.V + Oxidation (M)
				791.37	1580.73	1580.71	101	K.LTEWTSSNVMEER.K
				772.73	2315.16	2315.13	55	R.VTEQESKPVQMMYQIGLFR.V + 2 Oxidation (M)
				826.11	2475.31	2475.3	93	R.NVLQPSSVDSQTAMVLVNAIVFK.G + Oxidation (M)
				1022.51	3064.52	3064.5	108	K.VHHANENIFYCPIAIMSALAMVYLGAK.D + 2 Oxidation (M)
13	Ovotransferrin	gi|1351295	10	Observed	Mr(expt)	Mr(calc)	Score	Peptide
				474.76	947.52	947.51	60	R.ISLTCVQK.A
				481.25	960.49	960.49	39	R.IQWCAVGK.D
				492.25	982.49	982.48	35	K.ATYLDCIK.A
				524.27	1046.52	1046.52	28	K.YFGYTGALR.C
				618.78	1235.55	1235.55	48	R.WCTISSPEEK.K
				667.35	1332.68	1332.65	56	R.IQWCAVGKDEK.S
				821.42	1640.82	1640.79	20	K.FFSASCVPGATIEQK.L
				1012.48	2022.95	2022.92	97	R.WSVVSNGDVECTVVDETK.D
				885.1	2652.28	2652.24	95	R.WSVVSNGDVECTVVDETKDCIIK.I
13	Ovotransferrin	gi|3024757	6	Observed	Mr(expt)	Mr(calc)	Score	Peptide
				481.25	960.49	960.49	39	K.IQWCAVGK.D
				492.25	982.49	982.48	35	K.ATYLDCIK.A
				667.35	1332.68	1332.65	56	K.IQWCAVGKDEK.S
				821.42	1640.82	1640.79	20	K.FFSASCVPGATIEQK.L
				1012.48	2022.95	2022.92	12	R.WSVVSNGEVECTILDDNK.D + Oxidation (C)
13	Chain A, Chicken Egg-White Lysozyme Core Mutants	gi|157831883	1	Observed	Mr(expt)	Mr(calc)	Score	Peptide
				877.44	1752.87	1752.83	131	R.NTDGSTDYGLLQINSR.W
13	ovoinhibitor precursor	gi|71895337	2	Observed	Mr(expt)	Mr(calc)	Score	Peptide
				882.41	1762.8	1762.78	85	R.QEIPEIDCDQYPTR.K
				1028.81	3083.42	3083.32	36	R.ILSPVCGTDGFTYDNECGICAHNAEQR.T
13	protein TENP [Gallus gallus]	gi|46048814	1	Observed	Mr(expt)	Mr(calc)	Score	Peptide
				819.97	1637.93	1637.91	87	K.VADLWLSVIPEAGLR.L
14	ovotransferrin precursor	gi|45385813	10	Observed	Mr(expt)	Mr(calc)	Score	Peptide
				445.23	888.45	888.43	48	R.DLTQQER.I
				474.7	947.39	947.51	54	R.ISLTCVQK.A
				492.25	982.49	982.48	35	K.ATYLDCIK.A
				524.27	1046.52	1046.52	46	K.YFGYTGALR.C
				618.77	1235.53	1235.55	52	R.WCTISSPEEK.K
				822.4	1642.79	1642.78	101	R.LCQLCQGSGGIPPEK.C
				878.77	2633.3	2633.3	93	K.AIANNEADAISLDGGQVFEAGLAPYK.L
				885.1	2652.27	2652.24	111	R.WSVVSNGDVECTVVDETKDCIIK.I
14	ovotransferrin CC type	gi|71274077	10	Observed	Mr(expt)	Mr(calc)	Score	Peptide
				445.23	888.45	888.43	48	R.DLTQQER.I
				466.76	931.51	931.52	44	R.IALTCVQK.A
				492.25	982.49	982.48	35	K.ATYLDCIK.A
				524.27	1046.52	1046.52	46	K.YFGYTGALR.C
				618.77	1235.53	1235.55	52	R.WCTISSPEEK.K
				822.4	1642.79	1642.78	101	R.LCQLCQGSGGIPPEK.C
				878.77	2633.3	2633.3	93	K.AIANNEADAISLDGGQVFEAGLAPYK.L
				885.1	2652.27	2652.24	111	R.WSVVSNGDVECTVVDETKDCIIK.I
14	Ovalbumin	gi|129293	7	Observed	Mr(expt)	Mr(calc)	Score	Peptide
				524.58	1570.73	1570.71	63	K.AFKDEDTQAMPFR.V + Oxidation (M)
				772.72	2315.15	2315.13	33	R.VTEQESKPVQMMYQIGLFR.V + 2 Oxidation (M)
				1238.66	2475.32	2475.3	82	R.NVLQPSSVDSQTAMVLVNAIVFK.G + Oxidation (M)
				767.17	3064.65	3064.5	84	K.VHHANENIFYCPIAIMSALAMVYLGAK.D + 2 Oxidation (M)
14	protein TENP [Gallus gallus]	gi|46048814	3	Observed	Mr(expt)	Mr(calc)	Score	Peptide
				819.97	1637.92	1637.91	90	K.VADLWLSVIPEAGLR.L
				967.5	2899.49	2899.46	61	R.AALLEELFLAPVCQQVPAWMDDVLR.E + Oxidation (M)
14	Ovomucoid	gi|124757	2	Observed	Mr(expt)	Mr(calc)	Score	Peptide
				544.8	1087.59	1087.56	71	K.VEQGASVDKR.H
				881.04	2640.09	2640.14	25	R.AFNPVCGTDGVTYDNECLLCAHK.V
14	Chain A, Chicken Egg-White Lysozyme Core Mutants	gi|157831883	1	Observed	Mr(expt)	Mr(calc)	Score	Peptide
				877.43	1752.84	1752.83	95	R.NTDGSTDYGLLQINSR.W
14	ovalbumin-related protein Y	gi|71897377	1	Observed	Mr(expt)	Mr(calc)	Score	Peptide

				676.79	1351.57	1351.56	52	K.FCFDVFNEMK.V + Oxidation (M)
15	Ovotransferrin	gi|1351295	11	Observed	Mr(expt)	Mr(calc)	Score	Peptide
				520.76	1039.5	1039.49	30	R.RANVMDYR.E + Oxidation (M)
				524.27	1046.53	1046.52	43	K.YFGYTGALR.C
				534.26	1066.5	1066.49	67	K.AQSDFGVDTK.S
				601.31	1200.61	1200.59	69	K.SDFHLFGPPGK.K
				665.36	1328.71	1328.69	37	K.SDFHLFGPPGKK.D
				718.88	1435.75	1435.73	121	K.KGTEFTVNDLQGK.T
				512.29	1533.85	1533.84	77	R.SAGWNIPIGTLLHR.G
				980.49	1958.96	1958.96	84	R.GAIEWEGIESGSVEQAVAK.F
				683.37	2047.09	2047.05	56	R.ECNLAEVPTHAVVVRPEK.A
15	ovotransferrin BB type	gi|71274075	11	Observed	Mr(expt)	Mr(calc)	Score	Peptide
				520.76	1039.5	1039.49	30	R.RANVMDYR.E + Oxidation (M)
				524.27	1046.53	1046.52	43	K.YFGYTGALR.C
				534.26	1066.5	1066.49	-50	K.AQSDFGVDTK.S
				534.26	1066.5	1066.49	67	K.AQSDFGVDTK.S
				601.31	1200.61	1200.59	69	K.SDFHLFGPPGK.K
				665.36	1328.71	1328.69	37	K.SDFHLFGPPGKK.D
				718.88	1435.75	1435.73	121	K.KGTEFTVNDLQGK.T
				512.29	1533.85	1533.84	77	R.SAGWNIPIGTLIHR.G
				980.49	1958.96	1958.96	84	R.GAIEWEGIESGSVEQAVAK.F
				683.37	2047.09	2047.05	56	R.ECNLAEVPTHAVVVRPEK.A
15	Ovalbumin	gi|129293	6	Observed	Mr(expt)	Mr(calc)	Score	Peptide
				624.33	1246.64	1246.62	39	R.ADHPFLFCIK.H
				673.38	1344.75	1344.73	73	K.HIATNAVLFFGR.C
				1238.67	2475.32	2475.3	86	R.NVLQPSSVDSQTAMVLVNAIVFK.G + Oxidation (M)
				767.14	3064.53	3064.5	82	K.VHHANENIFYCPIAIMSALAMVYLGAK.D + 2 Oxidation (M)
15	protein TENP [Gallus gallus]	gi|46048814	1	Observed	Mr(expt)	Mr(calc)	Score	Peptide
				819.98	1637.94	1637.91	57	K.VADLWLSVIPEAGLR.L
15	ovalbumin-related protein Y	gi|71897377	1	Observed	Mr(expt)	Mr(calc)	Score	Peptide
				706.87	1411.73	1411.72	49	R.YNPTNAILFFGR.Y
16	Ovotransferrin	gi|1351295	7	Observed	Mr(expt)	Mr(calc)	Score	Peptide
				474.77	947.52	947.51	41	R.ISLTCVQK.A
				524.28	1046.54	1046.52	39	K.YFGYTGALR.C
				529.3	1056.58	1056.59	49	K.FYTVISSLK.T
				618.78	1235.55	1235.55	50	R.WCTISSPEEK.K
				873.97	1745.93	1745.92	70	K.EFLGDKFYTVISSLK.T
				1008.45	2014.88	2014.88	84	K.TCNPSDILQMCSFLEGK.- + Oxidation (M)
16	Lysozyme	gi|126608	4	Observed	Mr(expt)	Mr(calc)	Score	Peptide
				497.2	992.38	992.39	27	R.WWCNDGR.T
				877.43	1752.84	1752.83	130	R.NTDGSTDYGILQINSR.W
				836.74	2507.2	2507.18	71	R.NLCNIPCSALLSSDITASVNCAK.K
				879.44	2635.29	2635.28	14	R.NLCNIPCSALLSSDITASVNCAKK.I
16	Lysozyme	gi|742827	4	Observed	Mr(expt)	Mr(calc)	Score	Peptide
				497.2	992.38	992.39	27	R.WWCNDGR.T
				877.43	1752.84	1752.83	130	R.NTDGSTDYGILQINSR.W
				836.74	2507.2	2507.18	58	R.NLCNIPCSALLSSDTIASVNCAK.K
				879.44	2635.29	2635.28	14	R.NLCNIPCSALLSSDTIASVNCAKK.I
16	Chain A, Im Mutant Of Lysozyme	gi|15988033	4	Observed	Mr(expt)	Mr(calc)	Score	Peptide
				497.2	992.38	992.39	27	R.WWCNDGR.T
				877.43	1752.84	1752.83	130	R.NTDGSTDYGILQINSR.W
				836.74	2507.2	2507.06	62	R.NLCNMPCSALLSSDITASVNCAK.K + Dioxidation (M); 2 Dioxidation (C)
16	Chain A, Chicken Egg-White Lysozyme Core Mutants	gi|157831883	2	Observed	Mr(expt)	Mr(calc)	Score	Peptide
				497.2	992.38	992.39	27	R.WWCNDGR.T
				877.43	1752.84	1752.83	130	R.NTDGSTDYGLLQINSR.W
16	unnamed protein product [Gallus gallus]	gi|63052	6	Observed	Mr(expt)	Mr(calc)	Score	Peptide
				1022.5	3064.49	3064.5	104	K.VHHANENIFYCPIAIMSALAMVYLGAK.D + 2 Oxidation (M)
17	Chain A, Aluminum-Bound Ovotransferrin	gi|83754919	24	Observed	Mr(expt)	Mr(calc)	Score	Peptide
				402.72	803.43	803.41	36	K.KCNNLR.D
				434.72	867.43	867.39	46	R.ANVMDYR.E
				445.23	888.45	888.43	45	R.DLTQQER.I
				457.77	913.52	913.53	36	K.IRDLLER.Q
				492.25	982.48	982.48	39	K.ATYLDCIK.A
				512.76	1023.5	1023.49	38	R.RANVMDYR.E
				529.29	1056.57	1056.59	49	K.FYTVISSLK.T
				618.78	1235.55	1235.55	53	R.WCTISSPEEK.K
				455.56	1363.67	1363.64	52	R.WCTISSPEEKK.C
				821.4	1640.78	1640.79	84	K.FFSASCVPGATIEQK.L

				873.93	1745.84	1745.92	114	K.EFLGDKFYTVISSLK.T
				980.49	1958.96	1958.96	28	R.GAIEWEGIESGSVEQAVAK.F
				1008.44	2014.87	2014.88	128	K.TCNPSDILQMCSFLEGK.- + Oxidation (M)
				683.35	2047.02	2047.05	71	R.ECNLAEVPTHAVVVRPEK.A
				878.78	2633.31	2633.3	49	K.AIANNEADAISLDGGQVFEAGLAPYK.L
17	ovotransferrin CC type	gi|71274077	22	Observed	Mr(expt)	Mr(calc)	Score	Peptide
				402.72	803.43	803.41	36	K.KCNNLR.D
				434.72	867.43	867.39	46	R.ANVMDYR.E
				445.23	888.45	888.43	45	R.DLTQQER.I
				457.77	913.52	913.53	36	K.IRDLLER.Q
				466.76	931.51	931.52	59	R.IALTCVQK.A
				492.25	982.48	982.48	39	K.ATYLDCIK.A
				512.76	1023.5	1023.49	38	R.RANVMDYR.E
				618.78	1235.55	1235.55	53	R.WCTISSPEEK.K
				455.56	1363.67	1363.64	52	R.WCTISSPEEKK.C
				821.4	1640.78	1640.79	84	K.FFSASCVPGATIEQK.L
				980.49	1958.96	1958.96	28	R.GAIEWEGIESGSVEQAVAK.F
				1008.44	2014.87	2014.88	128	K.TCNPSDILQMCSFLEGK.- + Oxidation (M)
				683.35	2047.02	2047.05	71	R.ECNLAEVPTHAVVVRPEK.A
				878.78	2633.31	2633.3	49	K.AIANNEADAISLDGGQVFEAGLAPYK.L
17	Lysozyme	gi|126608	5	Observed	Mr(expt)	Mr(calc)	Score	Peptide
				437.78	873.54	873.41	46	R.HGLDNYR.G
				663.32	1324.63	1324.62	66	R.GYSLGNWVCAAK.F
				714.83	1427.65	1427.64	101	K.FESNFNTQATNR.N
				877.43	1752.84	1752.83	129	R.NTDGSTDYGILQINSR.W
				836.74	2507.19	2507.18	25	R.NLCNIPCSALLSSDITASVNCAK.K
17	Chain A, Im Mutant Of Lysozyme	gi|15988033	5	Observed	Mr(expt)	Mr(calc)	Score	Peptide
				437.78	873.54	873.41	46	R.HGLDNYR.G
				663.32	1324.63	1324.62	66	R.GYSLGNWVCAAK.F
				714.83	1427.65	1427.64	101	K.FESNFNTQATNR.N
				877.43	1752.84	1752.83	129	R.NTDGSTDYGILQINSR.W
				836.74	2507.19	2507.05	16	R.NLCNMPCSALLSSDITASVNCAK.K + Oxidation (C); Sulfo (C)
17	Chain A,Chicken Egg-White Lysozyme Core Mutants	gi|157831883	4	Observed	Mr(expt)	Mr(calc)	Score	Peptide
				437.78	873.54	873.41	46	R.HGLDNYR.G
				663.32	1324.63	1324.62	66	R.GYSLGNWVCAAK.F
				714.83	1427.65	1427.64	101	K.FESNFNTQATNR.N
				877.43	1752.84	1752.83	129	R.NTDGSTDYGLLQINSR.W
17	Lysozyme	gi|742827	3	Observed	Mr(expt)	Mr(calc)	Score	Peptide
				663.32	1324.63	1324.62	66	R.GYSLGNWVCAAK.F
				877.43	1752.84	1752.83	129	R.NTDGSTDYGILQINSR.W
				836.74	2507.19	2507.18	17	R.NLCNIPCSALLSSDTIASVNCAK.K
17	unnamed protein product [Gallus gallus]	gi|63052	3	Observed	Mr(expt)	Mr(calc)	Score	Peptide
				1022.55	3064.61	3064.5	69	K.VHHANENIFYCPIAIMSALAMVYLGAK.D + 2 Oxidation (M)
17	protein TENP [Gallus gallus]	gi|46048814	1	Observed	Mr(expt)	Mr(calc)	Score	Peptide
				819.97	1637.92	1637.91	51	K.VADLWLSVIPEAGLR.L
18	Hep21 protein [Gallus gallus]	gi|45383131	2	Observed	Mr(expt)	Mr(calc)	Score	Peptide
				789.8	1577.58	1577.58	73	R.YSCCETDLCNEK.W
				995.95	1989.88	1989.9	116	K.VTLYYQQGCTSALNCGR.E
18	Ovalbumin	gi|129293	4	Observed	Mr(expt)	Mr(calc)	Score	Peptide
				844.43	1686.85	1686.83	64	R.GGLEPINFQTAADQAR.E
				1230.66	2459.31	2459.31	31	R.NVLQPSSVDSQTAMVLVNAIVFK.G
				767.17	3064.65	3064.5	78	K.VHHANENIFYCPIAIMSALAMVYLGAK.D + 2 Oxidation (M)
18	Ovotransferrin	gi|1351295	3	Observed	Mr(expt)	Mr(calc)	Score	Peptide
				524.27	1046.52	1046.52	39	K.YFGYTGALR.C
				821.41	1640.8	1640.79	49	K.FFSASCVPGATIEQK.L
				873.97	1745.94	1745.92	54	K.EFLGDKFYTVISSLK.T
18	Chain A, Chicken Egg-White Lysozyme Core Mutants	gi|157831883	1	Observed	Mr(expt)	Mr(calc)	Score	Peptide
				877.42	1752.83	1752.83	126	R.NTDGSTDYGLLQINSR.W
19	Chain A, Aluminum-Bound Ovotransferrin	gi|83754919	79	Observed	Mr(expt)	Mr(calc)	Score	Peptide
				404.18	806.35	806.35	35	K.TCNWAR.V
				416.74	831.46	831.42	33	K.NKADWAK.N
				434.72	867.42	867.39	44	R.ANVMDYR.E
				445.23	888.44	888.43	46	R.DLTQQER.I
				447.27	892.52	892.52	52	R.VAAHAVVAR.D
				457.75	913.49	913.53	39	K.IRDLLER.Q
				474.77	947.53	947.51	59	R.ISLTCVQK.A
				475.26	948.51	948.51	58	K.DSAIMLKR.V + Oxidation (M)
				481.24	960.47	960.49	40	R.IQWCAVGK.D

				492.27	982.54	982.48	35	K.ATYLDCIK.A
				497.26	992.51	992.5	48	K.RFGVNGSEK.S
				512.76	1023.5	1023.49	40	R.RANVMDYR.E
				518.24	1034.47	1034.54	81	K.DGKGDVAFVK.H
				524.29	1046.56	1046.52	44	K.YFGYTGALR.C
				529.28	1056.55	1056.59	49	K.FYTVISSLK.T
				534.26	1066.5	1066.49	42	K.AQSDFGVDTK.S
				546.82	1091.63	1091.62	53	K.DLLFKDLTK.C
				594.35	1186.68	1186.7	82	K.DPVLKDLLFK.D
				597.24	1192.47	1192.49	47	K.FMMFESQNK.D + 2 Oxidation (M)
				601.29	1200.56	1200.59	58	K.SDFHLFGPPGK.K
				602.29	1202.56	1202.57	49	K.DSNVNWNNLK.G
				612.29	1222.57	1222.62	59	K.VEDIWSFLSK.A
				618.74	1235.47	1235.55	53	R.WCTISSPEEK.K
				654.83	1307.64	1307.64	60	K.GTEFTVNDLQGK.T
				443.9	1328.69	1328.69	45	K.SDFHLFGPPGKK.D
				667.34	1332.66	1332.65	59	R.IQWCAVGKDEK.S
				677.34	1352.66	1352.63	73	K.HTTVNENAPDQK.D
				455.6	1363.76	1363.64	55	R.WCTISSPEEKK.C
				704.82	1407.63	1407.62	53	K.SKFMMFESQNK.D + 2 Oxidation (M)
				705.39	1408.76	1408.76	72	K.DLLFKDSAIMLK.R + Oxidation (M)
				718.86	1435.71	1435.73	134	K.KGTEFTVNDLQGK.T
				735.32	1468.63	1468.65	79	K.DEYELLCLDGSR.Q
				512.28	1533.82	1533.84	72	R.SAGWNIPIGTLIHR.G
				821.4	1640.78	1640.79	93	K.FFSASCVPGATIEQK.L
				826.92	1651.82	1651.83	45	K.TDERPASYFAVAVAR.K
				836.37	1670.72	1670.75	88	R.NAPYSGYSGAFHCLK.D
				873.92	1745.82	1745.92	112	K.EFLGDKFYTVISSLK.T
				603.95	1808.84	1808.85	69	K.FMMFESQNKDLLFK.D + 2 Oxidation (M)
				913.89	1825.76	1825.8	109	K.NLQMDDFELLCTDGR.R
				980.47	1958.93	1958.96	117	R.GAIEWEGIESGSVEQAVAK.F
				666.94	1997.81	1997.89	44	K.NLQMDDFELLCTDGRR.A + Oxidation (M)
				1008.39	2014.77	2014.88	118	K.TCNPSDILQMCSFLEGK.- + Oxidation (M)
				683.34	2047	2047.05	69	R.ECNLAEVPTHAVVVRPEK.A
				850.67	2549	2549.02	46	R.TGTCNFDEYFSEGCAPGSPPNSR.L
				878.77	2633.3	2633.3	104	K.AIANNEADAISLDGGQVFEAGLAPYK.L
				885.08	2652.22	2652.24	95	R.WSVVSNGDVECTVVDETKDCIIK.I
				935.42	2803.24	2803.27	112	K.HTTVNENAPDQKDEYELLCLDGSR.Q
				936.14	2805.39	2805.37	92	K.GEADAVALDGGLVYTAGVCGLVPVMAER.Y + Oxidation (M)
				1065.54	3193.59	3193.58	102	K.IMKGEADAVALDGGLVYTAGVCGLVPVMAER.Y + 2 Oxidation (M)
19	Ovotransferrin	gi|1351295	77	Observed	Mr(expt)	Mr(calc)	Score	Peptide
				404.18	806.35	806.35	35	K.TCNWAR.V
				416.74	831.46	831.42	33	K.NKADWAK.N
				419.22	836.42	836.4	45	R.FGVNGSEK.S
				434.72	867.42	867.39	44	R.ANVMDYR.E
				445.23	888.44	888.43	46	R.DLTQQER.I
				447.27	892.52	892.52	52	R.VAAHAVVAR.D
				457.75	913.49	913.53	39	K.IRDLLER.Q
				474.77	947.53	947.51	59	R.ISLTCVQK.A
				475.26	948.51	948.51	58	K.DSAIMLKR.V + Oxidation (M)
				481.24	960.47	960.49	40	R.IQWCAVGK.D
				492.27	982.54	982.48	35	K.ATYLDCIK.A
				497.26	992.51	992.5	48	K.RFGVNGSEK.S
				512.76	1023.5	1023.49	40	R.RANVMDYR.E
				518.24	1034.47	1034.54	81	K.DGKGDVAFVK.H
				524.29	1046.56	1046.52	44	K.YFGYTGALR.C
				529.28	1056.55	1056.59	49	K.FYTVISSLK.T
				534.26	1066.5	1066.49	42	K.AQSDFGVDTK.S
				546.82	1091.63	1091.62	53	K.DLLFKDLTK.C
				594.35	1186.68	1186.7	82	K.DPVLKDLLFK.D
				597.24	1192.47	1192.49	47	K.FMMFESQNK.D + 2 Oxidation (M)
				601.29	1200.56	1200.59	58	K.SDFHLFGPPGK.K
				602.29	1202.56	1202.57	49	K.DSNVNWNNLK.G
				612.29	1222.57	1222.62	59	K.VEDIWSFLSK.A
				618.74	1235.47	1235.55	53	R.WCTISSPEEK.K
				654.83	1307.64	1307.64	60	K.GTEFTVNDLQGK.T
				443.9	1328.69	1328.69	45	K.SDFHLFGPPGKK.D
				667.34	1332.66	1332.65	59	R.IQWCAVGKDEK.S
				677.34	1352.66	1352.63	73	K.HTTVNENAPDQK.D
				455.6	1363.76	1363.64	55	R.WCTISSPEEKK.C
				704.82	1407.63	1407.62	53	K.SKFMMFESQNK.D + 2 Oxidation (M)
				705.39	1408.76	1408.76	72	K.DLLFKDSAIMLK.R + Oxidation (M)
				718.86	1435.71	1435.73	134	K.KGTEFTVNDLQGK.T
				735.32	1468.63	1468.65	79	K.DEYELLCLDGSR.Q
				512.28	1533.82	1533.84	72	R.SAGWNIPIGTLLHR.G
				821.4	1640.78	1640.79	93	K.FFSASCVPGATIEQK.L
				826.92	1651.82	1651.83	45	K.TDERPASYFAVAVAR.K
				836.37	1670.72	1670.75	88	R.NAPYSGYSGAFHCLK.D
				873.92	1745.82	1745.92	112	K.EFLGDKFYTVISSLK.T
				603.95	1808.84	1808.85	69	K.FMMFESQNKDLLFK.D + 2 Oxidation (M)
				913.89	1825.76	1825.8	109	K.NLQMDDFELLCTDGR.R
				980.47	1958.93	1958.96	117	R.GAIEWEGIESGSVEQAVAK.F
				666.94	1997.81	1997.89	44	K.NLQMDDFELLCTDGRR.A + Oxidation (M)
				1008.39	2014.77	2014.88	118	K.TCNPSDILQMCSFLEGK.- + Oxidation (M)

				683.34	2047	2047.05	69	R.ECNLAEVPTHAVVVRPEK.A
				850.67	2549	2549.02	46	R.TGTCNFDEYFSEGCAPGSPPNSR.L
				885.08	2652.22	2652.24	95	R.WSVVSNGDVECTVVDETKDCIIK.I
				935.42	2803.24	2803.27	112	K.HTTVNENAPDQKDEYELLCLDGSR.Q
				936.14	2805.39	2805.37	92	K.GEADAVALDGGLVYTAGVCGLVPVMAER.Y + Oxidation (M)
				1065.54	3193.59	3193.58	102	K.IMKGEADAVALDGGLVYTAGVCGLVPVMAER.Y + 2 Oxidation (M)
19	Chain A, Ovotransferrin, N-Terminal Lobe, Holo Form	gi|14719680	36	Observed	Mr(expt)	Mr(calc)	Score	Peptide
				404.18	806.35	806.35	35	K.TCNWAR.V
				445.23	888.44	888.43	46	R.DLTQQER.I
				447.27	892.52	892.52	52	R.VAAHAVVAR.D
				474.77	947.53	947.51	59	R.ISLTCVQK.A
				475.26	948.51	948.51	58	K.DSAIMLKR.V + Oxidation (M)
				492.27	982.54	982.48	35	K.ATYLDCIK.A
				518.24	1034.47	1034.54	81	K.DGKGDVAFVK.H
				534.26	1066.5	1066.49	42	K.AQSDFGVDTK.S
				594.35	1186.68	1186.7	82	K.DPVLKDLLFK.D
				601.29	1200.56	1200.59	58	K.SDFHLFGPPGK.K
				612.29	1222.57	1222.62	59	K.VEDIWSFLSK.A
				618.74	1235.47	1235.55	53	R.WCTISSPEEK.K
				654.83	1307.64	1307.64	60	K.GTEFTVNDLQGK.T
				443.9	1328.69	1328.69	45	K.SDFHLFGPPGKK.D
				677.34	1352.66	1352.63	73	K.HTTVNENAPDQK.D
				455.6	1363.76	1363.64	55	R.WCTISSPEEKK.C
				705.39	1408.76	1408.76	72	K.DLLFKDSAIMLK.R + Oxidation (M)
				718.86	1435.71	1435.73	134	K.KGTEFTVNDLQGK.T
				735.32	1468.63	1468.65	79	K.DEYELLCLDGSR.Q
				512.28	1533.82	1533.84	72	R.SAGWNIPIGTLLHR.G
				821.4	1640.78	1640.79	93	K.FFSASCVPGATIEQK.L
				836.37	1670.72	1670.75	88	R.NAPYSGYSGAFHCLK.D
				980.47	1958.93	1958.96	117	R.GAIEWEGIESGSVEQAVAK.F
				883.47	2647.37	2647.31	50	K.AIANNEADAITLDGGQVFEAGLAPYK.L
				935.42	2803.24	2803.27	112	K.HTTVNENAPDQKDEYELLCLDGSR.Q
19	Ovalbumin	gi|129293	9	Observed	Mr(expt)	Mr(calc)	Score	Peptide
				624.32	1246.63	1246.62	19	R.ADHPFLFCIK.H
				761.92	1521.82	1521.79	38	R.YPILPEYLQCVK.E
				786.37	1570.72	1570.71	57	K.AFKDEDTQAMPFR.V + Oxidation (M)
				799.36	1596.71	1596.71	104	K.LTEWTSSNVMEER.K + Oxidation (M)
				844.4	1686.79	1686.83	96	R.GGLEPINFQTAADQAR.E
				772.71	2315.11	2315.13	55	R.VTEQESKPVQMMYQIGLFR.V + 2 Oxidation (M)
				820.79	2459.34	2459.31	57	R.NVLQPSSVDSQTAMVLVNAIVFK.G
				1022.55	3064.64	3064.5	36	K.VHHANENIFYCPIAIMSALAMVYLGAK.D + 2 Oxidation (M)
20	Chain A, Aluminum-Bound Ovotransferrin	gi|83754919	99	Observed	Mr(expt)	Mr(calc)	Score	Peptide
				397.28	792.55	792.41	43	K.DSAIMLK.R + Oxidation (M)
				404.18	806.34	806.35	35	K.TCNWAR.V
				419.27	836.53	836.4	45	R.FGVNGSEK.S
				434.7	867.39	867.39	44	R.ANVMDYR.E
				445.23	888.44	888.43	41	R.DLTQQER.I
				447.25	892.49	892.52	51	R.VAAHAVVAR.D
				457.27	912.53	912.47	34	K.DQLTPSPR.E
				457.74	913.46	913.53	36	K.IRDLLER.Q
				474.69	947.36	947.51	32	R.ISLTCVQK.A
				492.28	982.54	982.48	34	K.ATYLDCIK.A
				494.75	987.48	987.46	56	K.TSCHTGLGR.S
				497.26	992.5	992.5	56	K.RFGVNGSEK.S
				512.75	1023.49	1023.49	32	R.RANVMDYR.E
				518.27	1034.53	1034.54	78	K.DGKGDVAFVK.H
				524.27	1046.53	1046.52	46	K.YFGYTGALR.C
				529.24	1056.48	1056.59	49	K.FYTVISSLK.T
				534.24	1066.47	1066.49	32	K.AQSDFGVDTK.S
				566.23	1130.45	1130.42	54	R.YDDESQCSK.T
				594.35	1186.69	1186.7	89	K.DPVLKDLLFK.D
				597.26	1192.5	1192.49	44	K.FMMFESQNK.D + 2 Oxidation (M)
				601.26	1200.51	1200.59	61	K.SDFHLFGPPGK.K
				612.31	1222.6	1222.62	59	K.VEDIWSFLSK.A
				618.77	1235.52	1235.55	53	R.WCTISSPEEK.K
				654.79	1307.56	1307.64	69	K.GTEFTVNDLQGK.T
				443.89	1328.64	1328.69	38	K.SDFHLFGPPGKK.D
				667.33	1332.65	1332.65	55	R.IQWCAVGKDEK.S
				455.55	1363.62	1363.64	51	R.WCTISSPEEKK.C
				696.82	1391.62	1391.62	71	K.SKFMMFESQNK.D + Oxidation (M)
				735.3	1468.59	1468.65	74	K.DEYELLCLDGSR.Q
				512.25	1533.72	1533.84	59	R.SAGWNIPIGTLIHR.G
				790.91	1579.81	1579.83	53	R.TAGWVIPMGLIHNR.T + Oxidation (M)
				821.4	1640.78	1640.79	77	K.FFSASCVPGATIEQK.L
				551.61	1651.82	1651.83	43	K.TDERPASYFAVAVAR.K
				836.33	1670.64	1670.75	87	R.NAPYSGYSGAFHCLK.D
				873.91	1745.8	1745.92	111	K.EFLGDKFYTVISSLK.T
				603.98	1808.92	1808.85	69	K.FMMFESQNKDLLFK.D + 2 Oxidation (M)
				913.85	1825.68	1825.8	104	K.NLQMDDFELLCTDGR.R

				980.48	1958.95	1958.96	124	R.GAIEWEGIESGSVEQAVAK.F
				666.97	1997.9	1997.89	53	K.NLQMDDFELLCTDGRR.A + Oxidation (M)
				1012.45	2022.9	2022.92	92	R.WSVVSNGDVECTVVDETK.D
				1016.42	2030.83	2030.87	20	K.TCNPSDILQMCSFLEGK.- + Dioxidation (M)
				512.8	2047.17	2047.05	42	R.ECNLAEVPTHAVVVRPEK.A
				878.77	2633.28	2633.3	120	K.AIANNEADAISLDGGQVFEAGLAPYK.L
				910.76	2729.27	2729.27	16	R.VPSLMDSQLYLGFEYYSAIQSMR.K + 2 Oxidation (M)
				936.12	2805.35	2805.37	119	K.GEADAVALDGGLVYTAGVCGLVPVMAER.Y + Oxidation (M)
				1065.52	3193.53	3193.58	91	K.IMKGEADAVALDGGLVYTAGVCGLVPVMAER.Y + 2 Oxidation (M)
20	ovotransferrin CC type	gi|71274077	94	Observed	Mr(expt)	Mr(calc)	Score	Peptide
				397.28	792.55	792.41	43	K.DSAIMLK.R + Oxidation (M)
				404.18	806.34	806.35	35	K.TCNWAR.V
				419.27	836.53	836.4	45	R.FGVNGSEK.S
				434.7	867.39	867.39	44	R.ANVMDYR.E
				445.23	888.44	888.43	41	R.DLTQQER.I
				447.25	892.49	892.52	51	R.VAAHAVVAR.D
				457.27	912.53	912.47	34	K.DQLTPSPR.E
				457.74	913.46	913.53	36	K.IRDLLER.Q
				492.28	982.54	982.48	34	K.ATYLDCIK.A
				494.75	987.48	987.46	56	K.TSCHTGLGR.S
				497.26	992.5	992.5	56	K.RFGVNGSEK.S
				512.75	1023.49	1023.49	32	R.RANVMDYR.E
				518.27	1034.53	1034.54	78	K.DGKGDVAFVK.H
				524.27	1046.53	1046.52	46	K.YFGYTGALR.C
				534.24	1066.47	1066.49	32	K.AQSDFGVDTK.S
				566.23	1130.45	1130.42	54	R.YDDESQCSK.T
				594.35	1186.69	1186.7	89	K.DPVLKDLLFK.D
				597.26	1192.5	1192.49	44	K.FMMFESQNK.D + 2 Oxidation (M)
				601.26	1200.51	1200.59	61	K.SDFHLFGPPGK.K
				612.31	1222.6	1222.62	59	K.VEDIWSFLSK.A
				618.77	1235.52	1235.55	53	R.WCTISSPEEK.K
				654.79	1307.56	1307.64	69	K.GTEFTVNDLQGK.T
				443.89	1328.64	1328.69	38	K.SDFHLFGPPGKK.D
				667.33	1332.65	1332.65	55	R.IQWCAVGKDEK.S
				455.55	1363.62	1363.64	51	R.WCTISSPEEKK.C
				696.82	1391.62	1391.62	71	K.SKFMMFESQNK.D + Oxidation (M)
				735.3	1468.59	1468.65	74	K.DEYELLCLDGSR.Q
				512.25	1533.72	1533.84	59	R.SAGWNIPIGTLIHR.G
				790.91	1579.81	1579.83	53	R.TAGWVIPMGLIHNR.T + Oxidation (M)
				821.4	1640.78	1640.79	77	K.FFSASCVPGATIEQK.L
				551.61	1651.82	1651.83	43	K.TDERPASYFAVAVAR.K
				836.33	1670.64	1670.75	87	R.NAPYSGYSGAFHCLK.D
				603.98	1808.92	1808.85	69	K.FMMFESQNKDLLFK.D + 2 Oxidation (M)
				913.85	1825.68	1825.8	104	K.NLQMDDFELLCTDGR.R
				980.48	1958.95	1958.96	124	R.GAIEWEGIESGSVEQAVAK.F
				666.97	1997.9	1997.89	53	K.NLQMDDFELLCTDGRR.A + Oxidation (M)
				1012.45	2022.9	2022.92	92	R.WSVVSNGDVECTVVDETK.D
				1016.42	2030.83	2030.87	20	K.TCNPSDILQMCSFLEGK.- + Dioxidation (M)
				512.8	2047.17	2047.05	42	R.ECNLAEVPTHAVVVRPEK.A
				858	2570.99	2570.97	88	R.TGTCNFNEYFSEGCAPGSPPNSR.L + Sulfo (C)
				878.77	2633.28	2633.3	120	K.AIANNEADAISLDGGQVFEAGLAPYK.L
				910.76	2729.27	2729.27	16	R.VPSLMDSQLYLGFEYYSAIQSMR.K + 2 Oxidation (M)
				936.12	2805.35	2805.37	119	K.GEADAVALDGGLVYTAGVCGLVPVMAER.Y + Oxidation (M)
20	Ovotransferrin	gi|1351295	96	Observed	Mr(expt)	Mr(calc)	Score	Peptide
				397.28	792.55	792.41	43	K.DSAIMLK.R + Oxidation (M)
				404.18	806.34	806.35	35	K.TCNWAR.V
				419.27	836.53	836.4	45	R.FGVNGSEK.S
				434.7	867.39	867.39	44	R.ANVMDYR.E
				445.23	888.44	888.43	41	R.DLTQQER.I
				447.25	892.49	892.52	51	R.VAAHAVVAR.D
				457.27	912.53	912.47	34	K.DQLTPSPR.E
				457.74	913.46	913.53	36	K.IRDLLER.Q
				474.69	947.36	947.51	32	R.ISLTCVQK.A
				492.28	982.54	982.48	34	K.ATYLDCIK.A
				494.75	987.48	987.46	56	K.TSCHTGLGR.S
				497.26	992.5	992.5	56	K.RFGVNGSEK.S
				512.75	1023.49	1023.49	32	R.RANVMDYR.E
				518.27	1034.53	1034.54	78	K.DGKGDVAFVK.H
				524.27	1046.53	1046.52	46	K.YFGYTGALR.C
				529.24	1056.48	1056.59	49	K.FYTVISSLK.T
				534.24	1066.47	1066.49	32	K.AQSDFGVDTK.S
				566.23	1130.45	1130.42	54	R.YDDESQCSK.T
				594.35	1186.69	1186.7	89	K.DPVLKDLLFK.D
				597.26	1192.5	1192.49	44	K.FMMFESQNK.D + 2 Oxidation (M)
				601.26	1200.51	1200.59	61	K.SDFHLFGPPGK.K
				612.31	1222.6	1222.62	59	K.VEDIWSFLSK.A
				618.77	1235.52	1235.55	53	R.WCTISSPEEK.K
				654.79	1307.56	1307.64	69	K.GTEFTVNDLQGK.T
				443.89	1328.64	1328.69	38	K.SDFHLFGPPGKK.D
				667.33	1332.65	1332.65	55	R.IQWCAVGKDEK.S

				455.55	1363.62	1363.64	51	R.WCTISSPEEKK.C
				696.82	1391.62	1391.62	71	K.SKFMMFESQNK.D + Oxidation (M)
				735.3	1468.59	1468.65	74	K.DEYELLCLDGSR.Q
				512.25	1533.72	1533.84	59	R.SAGWNIPIGTLLHR.G
				790.91	1579.81	1579.83	53	R.TAGWVIPMGLIHNR.T + Oxidation (M)
				821.4	1640.78	1640.79	77	K.FFSASCVPGATIEQK.L
				551.61	1651.82	1651.83	43	K.TDERPASYFAVAVAR.K
				836.33	1670.64	1670.75	87	R.NAPYSGYSGAFHCLK.D
				873.91	1745.8	1745.92	111	K.EFLGDKFYTVISSLK.T
				603.98	1808.92	1808.85	69	K.FMMFESQNKDLLFK.D + 2 Oxidation (M)
				913.85	1825.68	1825.8	104	K.NLQMDDFELLCTDGR.R
				980.48	1958.95	1958.96	124	R.GAIEWEGIESGSVEQAVAK.F
				666.97	1997.9	1997.89	53	K.NLQMDDFELLCTDGRR.A + Oxidation (M)
				1012.45	2022.9	2022.92	92	R.WSVVSNGDVECTVVDETK.D
				1016.42	2030.83	2030.87	20	K.TCNPSDILQMCSFLEGK.- + Dioxidation (M)
				512.8	2047.17	2047.05	42	R.ECNLAEVPTHAVVVRPEK.A
				910.76	2729.27	2729.27	16	R.VPSLMDSQLYLGFEYYSAIQSMR.K + 2 Oxidation (M)
				936.12	2805.35	2805.37	119	K.GEADAVALDGGLVYTAGVCGLVPVMAER.Y + Oxidation (M)
				1065.52	3193.53	3193.58	91	K.IMKGEADAVALDGGLVYTAGVCGLVPVMAER.Y + 2 Oxidation (M)
20	Chain A, Ovotransferrin, N-Terminal Lobe, Holo Form	gi|14719680	46	Observed	Mr(expt)	Mr(calc)	Score	Peptide
				397.28	792.55	792.41	43	K.DSAIMLK.R + Oxidation (M)
				404.18	806.34	806.35	35	K.TCNWAR.V
				445.23	888.44	888.43	41	R.DLTQQER.I
				447.25	892.49	892.52	51	R.VAAHAVVAR.D
				474.69	947.36	947.51	32	R.ISLTCVQK.A
				492.28	982.54	982.48	34	K.ATYLDCIK.A
				494.75	987.48	987.46	56	K.TSCHTGLGR.S
				518.27	1034.53	1034.54	78	K.DGKGDVAFVK.H
				534.24	1066.47	1066.49	32	K.AQSDFGVDTK.S
				594.35	1186.69	1186.7	89	K.DPVLKDLLFK.D
				601.26	1200.51	1200.59	61	K.SDFHLFGPPGK.K
				612.31	1222.6	1222.62	59	K.VEDIWSFLSK.A
				618.77	1235.52	1235.55	53	R.WCTISSPEEK.K
				654.79	1307.56	1307.64	69	K.GTEFTVNDLQGK.T
				443.89	1328.64	1328.69	38	K.SDFHLFGPPGKK.D
				455.55	1363.62	1363.64	51	R.WCTISSPEEKK.C
				735.3	1468.59	1468.65	74	K.DEYELLCLDGSR.Q
				512.25	1533.72	1533.84	59	R.SAGWNIPIGTLLHR.G
				821.4	1640.78	1640.79	77	K.FFSASCVPGATIEQK.L
				836.33	1670.64	1670.75	87	R.NAPYSGYSGAFHCLK.D
				980.48	1958.95	1958.96	124	R.GAIEWEGIESGSVEQAVAK.F
				883.44	2647.3	2647.31	56	K.AIANNEADAITLDGGQVFEAGLAPYK.L
				910.76	2729.27	2729.27	16	R.VPSLMDSQLYLGFEYYSAIQSMR.- + 2 Oxidation (M)
20	Ovotransferrin	gi|3024757	16	Observed	Mr(expt)	Mr(calc)	Score	Peptide
				397.28	792.55	792.41	43	K.DSAIMLK.R + Oxidation (M)
				404.18	806.34	806.35	35	K.TCNWAR.V
				447.25	892.49	892.52	51	R.VAAHAVVAR.D
				492.28	982.54	982.48	34	K.ATYLDCIK.A
				494.75	987.48	987.46	56	K.TSCHTGLGR.S
				518.27	1034.53	1034.54	78	K.DGKGDVAFVK.H
				594.35	1186.69	1186.7	89	K.DPVLKDLLFK.D
				667.33	1332.65	1332.65	55	K.IQWCAVGKDEK.S
				735.3	1468.59	1468.65	74	K.DEYELLCLDGSR.Q
				512.25	1533.72	1533.84	59	R.SAGWNIPIGTLIHR.E
				821.4	1640.78	1640.79	77	K.FFSASCVPGATIEQK.L
				1012.45	2022.9	2022.92	8	R.WSVVSNGEVECTILDDNK.D + Oxidation (C)
20	Ovalbumin	gi|129293	2	Observed	Mr(expt)	Mr(calc)	Score	Peptide
				820.79	2459.35	2459.31	9	R.NVLQPSSVDSQTAMVLVNAIVFK.G
				767.13	3064.49	3064.5	46	K.VHHANENIFYCPIAIMSALAMVYLGAK.D + 2 Oxidation (M)

A3: Gel hardness, gumminess, cohesiveness, and resilience values with one standard deviation error

Sample	Hardness	Gumminess	Cohesiveness	Resilience
95°C	2329.73 ± 209.16	1565.76 ± 184.43	0.67 ± 0.04	0.43 ± 0.03
95°C pH 6	1105.30 ± 282.41	580.99 ± 108.85	0.53 ± 0.05	0.19 ± 0.03
600 MPa	403.54 ± 93.40	276.82 ± 93.75	0.69 ± 0.17	0.18 ± 0.08
600 MPa pH 6	723.99 ± 124.11	503.41 ± 78.90	0.70 ± 0.01	0.32 ± 0.02
800 MPa	1114.89 ± 118.16	912.79 ± 114.63	0.82 ± 0.03	0.52 ± 0.01
800 MPa pH 6	1498.45 ± 119.13	1003.12 ± 69.07	0.67 ± 0.01	0.31 ± 0.01

A4: Gel color values with one standard deviation error

Sample	L-value	a-value	b-value
95C	85.75 ± 0.48	-4.55 ± 0.33	6.50 ± 0.92
95C pH6	87.75 ± 1.24	-2.99 ± 0.32	9.24 ±0.70
600MPa	81.87 ± 0.56	-3.63 ± 0.33	4.99 ± 1.09
600MPa pH6	88.97 ± 0.51	-1.92 ± 0.35	7.19 ± 0.75
800MPa	82.46 ± 1.01	-4.10 ± 0.35	3.88 ± 0.73
800MPa pH6	90.67 ± 0.73	-1.75 ± 0.14	7.13 ± 0.58

A5: Foam overrun values with one standard deviation error.

Time (min)	0	5	10	15	30
Control	1.5 ± 0.1	1.3 ± 0.2	1.3 ± 0.2	1.2 ± 0.2	1.2 ± 0.2
pH 4.5	2.4 ± 0.1	2.1 ± 0.1	2.0 ± 0.1	2.0 ± 0.1	1.9 ± 0.1
pH 6.0	1.1 ± 0.3	0.9 ± 0.3	0.9 ± 0.3	0.9 ± 0.3	0.9 ± 0.3
600 MPa	1.8 ± 0.2	1.6 ± 0.2	1.5 ± 0.2	1.5 ± 0.2	1.5 ± 0.2
800 MPa	2.1 ± 0.3	1.8 ± 0.2	1.8 ± 0.2	1.7 ± 0.2	1.7 ± 0.2

A6: Foam stability values with one standard deviation error.

Time (min)	0	5	10	15	30
Control	66.0 ± 3.7	46.0 ± 2.8	42.0 ± 3.0	40.0 ± 2.4	36.0 ± 2.8
pH 4.5	84.2 ± 8.3	60.0 ± 11.2	50.8 ± 11.3	45.0 ± 12.3	35.8 ± 12.0
pH 6.0	42.0 ± 14.1	28.7 ± 8.0	26.7 ± 5.3	26.0 ± 6.4	23.3 ± 5.3
600 MPa	48.7 ± 6.1	28.7 ± 4.5	23.3 ± 3.3	21.3 ± 1.8	18.7 ± 3.0
800 MPa	64.0 ± 10.9	32.0 ± 5.1	29.3 ± 2.8	26.0 ± 2.8	22.7 ± 2.8